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Corresponding author

E. Maréchal, Laboratoire de Physiologie Cellulaire et Végétale; Unité mixe de recherche 5168 CNRS, CEA, INRA, Université Grenoble Alpes; Institut de Biosciences et Biotechnologies de Grenoble; CEA Grenoble; 17, rue des Martyrs; 38000, Grenoble, France; +33 438784985; eric.marechal@cea.fr

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Authors

Florian Chevalier[1], Laura Cuyas[2,3], Juliette Jouhet[1], Valérie Gros[1], Serge Chiarenza[2], David Secco[4], James Whelan[4], Khawla Seddiki[1], Maryse A. Block[1], Laurent Nussaume[2,*], Eric Marechal[1,*]

Affiliations

[1]Laboratoire de Physiologie Cellulaire et Végétale; Unité mixe de recherche 5168 CNRS, CEA, INRA, Université Grenoble Alpes; Institut de Biosciences et Biotechnologies de Grenoble; CEA Grenoble; 17, rue des Martyrs; 38000, Grenoble, France

[2]Laboratoire de Biologie Végétale et Microbiologie Environnementale, Unité mixte de recherche 7265 CNRS, CEA, Université Aix-Marseille; Institut de Biosciences et Biotechnologies d'Aix-Marseille; CEA Cadarache; 18108, Saint-Paul-lez-Durance, France.

[3] Centre Mondial de l'Innovation. Groupe Roullier.18 avenue Franklin Roosevelt, 35400 Saint-Malo, France.

[4] Department of Animal, Plant and Soil Sciences, Australian Research Council Centre of Excellence in Plant Energy Biology, School of Life Sciences, La Trobe University, Bundoora, Victoria, Australia.

Present affiliations

Florian Chevalier, Thermo Fisher Scientific Inc

***Corresponding author**

E. Maréchal, Laboratoire de Physiologie Cellulaire et Végétale; Unité mixe de recherche 5168 CNRS, CEA, INRA, Université Grenoble Alpes; Institut de Biosciences et Biotechnologies de Grenoble; CEA Grenoble; 17, rue des Martyrs; 38000, Grenoble, France; +33 438784985; eric.marechal@cea.fr

Abstract.

Jasmonic acid (JA) biosynthesis and signaling are activated in *Arabidopsis* cultivated in phosphate (Pi) deprived conditions. This activation occurs mainly in photosynthetic tissues and is less important in roots. In leaves, the enhanced biosynthesis of JA coincides with membrane glycerolipid remodeling triggered by the lack of Pi. We addressed the possible role of JA on the dynamics and magnitude of glycerolipid remodeling in response to Pi-deprivation and resupply. Based on combined analyses of gene expression, JA biosynthesis and glycerolipid remodeling in wild type Arabidopsis and in the *coi1-16* mutant, JA signaling seems important in the determination of the basal levels of phosphatidylcholine (PC), phosphatidic acid (PA), monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). JA impact on MGDG steady state level and fluctuations seem contradictory. In the *coi1-16* mutant, the steady state level of MGDG is higher, possibly due to a higher level of PA in the mutant, activating MGD1, and to an increased expression of *MGD3*. These results support a possible impact of JA in limiting the overall content of this lipid. Concerning lipid variations, upon Pi-deprivation, JA seems rather associated with a specific MGDG increase. Following Pi-resupply, whereas the expression of glycerolipid remodeling genes returns to basal level, JA biosynthesis and signaling genes are still upregulated, likely due to a JA-induced positive feedback remaining active. Distinct impacts on enzymes synthesizing MGDG, *i.e.* downregulating *MGD3*, possibly activating *MGD1* expression and limiting the activation of MGD1 via PA, might allow JA playing a role in a sophisticated fine tuning of galactolipid variations.

Keywords

glycerolipid remodeling, jasmonic acid, MGD1, MGD3, phosphate signaling.

Introduction.

Plants have to cope with frequent variations of nutrients in soils. Phosphorus, in the form of soluble inorganic phosphate (Pi), is one of the most limiting elements (Rellan-Alvarez et al. 2016). The response to Pi starvation has been extensively studied in *Arabidopsis thaliana*, a plant assimilating Pi mainly via PHT1 transporters (Mitsukawa et al. 1997; Muchhal et al. 1997; Nussaume et al. 2011; Okumura et al. 1998). A lack of Pi triggers changes at multiple organization levels and time scales, ranging from early biochemical and metabolic tuning of Pi allocation, to genome scale transcription reprogramming, leading to an integrated metabolic, physiological and developmental response (Chiou and Lin 2011; Plaxton and Tran 2011; Yang and Finnegan 2010; Zhang et al. 2014). Primary long term phenotypic changes include: the secretion of phosphatases in the soil to scavenge Pi from organic sources, an enhanced expression of Pi transporters, a reduced growth of primary root and shoot, an enhanced growth of lateral roots and root hairs, an accumulation of anthocyanins and an intense remodeling of membrane glycerolipids, saving Pi from phospholipids (Peret et al. 2011).

The major early effector in low Pi signaling is the transcription factor PHR1 (Phosphate starvation response 1) (Bustos et al. 2010; Misson et al. 2005; Misson et al. 2004; Rubio et al. 2001; Thibaud et al. 2010). Concerning the control of Pi incorporation, PHR1 triggers the expression of miR399 in the shoot of *Arabidopsis*, and this microRNA moves to the root where it represses the expression of *PHO2*, encoding a ubiquitin ligase involved in the degradation of PHO1 (Lin et al. 2008; Liu et al. 2014b; Pant et al. 2008) and of several members of Phosphate transporters PHT1 family (Huang et al. 2013). The PHO1 Pi transporter then accumulates in the root xylem parenchyma, and operates in the transfer of Pi up to the shoot (Hamburger et al. 2002; Liu et al. 2012).

A later remodeling of lipids, marked by a replacement of phospholipids by Pi-free plastid glycolipids, is also under the control of PHR1 (Bustos et al. 2010; Pant et al. 2015). In brief, a phospholipid breakdown is triggered in non-plastidial membranes, mostly at the level of phosphatidylcholine (PC), involving the NPC4 and NPC5 phospholipases C (Gaude et al. 2008; Nakamura et al. 2005) and the PLDζ1 and PLDζ2 phospholipases D (Cruz-Ramirez et al. 2006). The mechanisms involved in the decline of phosphatidylglycerol (PG) in the plastid is unknown. Concomitantly, the syntheses of phosphorus-free sulfoquinovosyldiacylglycerol (SQDG), monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) increase in plastids (Awai et al. 2001; Boudiere et al. 2014; Dormann and Benning 2002; Essigmann et al. 1998; Hartel and Benning 2000; Jouhet et al. 2004; Jouhet et al. 2003; Jouhet et al. 2007; Yu et al. 2002). In the thylakoids, a net PG-to-SQDG replacement occurs (Essigmann et al. 1998), whereas DGDG is exported from the plastid and relocates to the plasma membrane (Andersson et al. 2003) and tonoplast (Andersson et al. 2005), where a PC-to-DGDG

replacement occurs. DGDG also relocates to mitochondria (Jouhet et al. 2004; Michaud et al. 2016). Genes involved in this lipid remodeling, i.e. the SQDG synthase (*SQD2*), two MGDG synthase isoforms (*MGD2* and *MGD3*) and DGDG synthases (*DGD1* and *DGD2*), are therefore markers of the low Pi response and of the remodeling of lipids.

It was recently shown that Pi deficiency also induced the biosynthesis of jasmonic acid (JA) and its derivative JA-Isoleucine (JA-Ile), therefore activating the JA signaling pathway (Khan et al. 2016). The biosynthesis of JA relies on the supply of alpha-linolenic acid (C18:3) derived from MGDG and DGDG, converted into oxo-phytodienoic acyls (OPDA) *via* a plastid lipoxygenase/allene oxide synthase (LOX/AOS) pathway (Andreou et al. 2009). OPDA is exported to the peroxisome and then serves as precursor for the synthesis of JA (Andreou et al. 2009; Wasternack and Hause 2013). Based on qRT-PCR studies, a lack of Pi induced the expression of gene markers of JA biosynthesis (lipoxygenase 2, *LOX2*) and signaling (jasmonate zim-domain protein 10, *JAZ10*) (Khan et al. 2016). A puzzling question is that, although multiple studies had been performed to analyze the transcriptomic response of roots and leaves of *Arabidopsis* to low Pi (Bustos et al. 2010; Hammond et al. 2003; Misson et al. 2005; Morcuende et al. 2007; Muller et al. 2007; Woo et al. 2012), only one reported a change in the expression of genes involved in JA biosynthesis and signaling (Morcuende et al. 2007). It was also shown that *JAZ10* was initially not induced in the *phr1-1* mutant, but reached the wild type induction level after 10 days on Pi-deficient medium, thus indicating an early (but partial) control by PHR1. The *pho1-7* mutation (Khan et al. 2016) was introgressed into *Arabidopsis* lines impaired in JA biosynthesis (the *aos* mutant defective for AOS (Park et al. 2002)) and JA signaling (the *coi1-34* mutant affecting the level of the COI1, an F-box protein interacting with JA-Ile (Acosta et al. 2013; Yan et al. 2013; Yan et al. 2009)). In the obtained *pho1-7 aos* and *pho1-7 coi1-34* double mutants, the expression of the low-Pi responsive gene *MGD3* was significantly reduced compared to the *pho1-7* parent. These experiments suggest that JA could influence *MGD3* expression, either by a transcriptional activation of *MGD3* that would be additive with the upregulation in response to low Pi, or by amplifying the magnitude of *MGD3* upregulation by low-Pi. Other studies support a link between galactolipid and JA levels. Consistently with the low Pi-response, Methyl-JA was shown to upregulate *MGD1*, in a context of increased DGDG production (Taki et al. 2005). By contrast, a mutant with 90% reduction of DGDG level due to the impairement of *DGD1* also shows a JA overproduction (Lin et al. 2016). Altogether, these studies show that different lipid remodeling patterns, with opposite variations of galactolipid levels, coincide with an increase in JA, suggesting that a sophisticated system of regulation by this oxylipin might operate.

In the present study, the transcriptomic changes induced by Pi deprivation and resupply were re-examined, refining the kinetics of gene expression responses. Previous reports showing that JA biosynthesis and signaling were activated upon Pi starvation were confirmed (Khan et al. 2016), but

showed that the expression of corresponding genes following Pi-resupply was not necessarily tuned down back to basal levels. We also confirmed that the activation of JA biosynthesis was different in roots and shoots (Khan et al. 2016), correlating with different patterns of upregulation of JA biosynthesis and signaling genes. We then attempted to refine our understanding of the interplay between low-Pi response, JA signaling and the control of glycerolipid homeostasis.

Results and discussion.

Transcriptional response to phosphate variations reveals different patterns of genes involved in jasmonic acid metabolism and signaling in different organs.

Arabidopsis seedlings were grown on modified media supplemented with either 500 µM KH_2PO4 (High Pi or "HPi") or 200 µM KCl (Low Pi or "LPi"). For the analysis of Pi deprivation and resupply, seeds were cultivated in LPi conditions for 7 days and then transferred for 30 min, 1 h or 3 h either to LPi (LPi-LPi; "Pi-deprived") or HPi (LPi-HPi; "Pi-resupply"). Plants cultivated in HPi conditions for 7 days before transfer onto fresh HPi medium (HPi-HPi; "Pi-supplemented") acted as controls (Fig. 1). Roots and shoots of *Arabidopsis* grown under these Pi regimen were carefully cut and collected using a razor blade, RNA was extracted and used to generate RNA-seq libraries. We thus obtained gene expression profiles in roots (Supplementary Table S1) and shoots (Supplementary Table S2) of *Arabidopsis* for each Pi regimen.

Genes that were differentially expressed in Pi-supplemented and Pi-deprived conditions were determined. To that purpose, compiled RNA-seq data was obtained from 0.5, 1 and 3 hours following transfers from HPi to HPi (Pi-supplemented) and LPi to LPi (Pi-deprived), respectively, and used to compare transcript abundance in shoots and roots. Using the non-parametric NOISeq method (Zheng and Moriyama 2013), only genes with a stable expression for each Pi concentration could be considered, based on the NOISeq p-value cutoff set at 0.05. Differentially expressed genes were then selected based on a Log-fold-change ratio threshold, i.e. $|\text{Log2FC}| > 1$.

In roots, 494 genes were down-regulated and 879 were up-regulated in low-Pi condition (Supplementary Table S3). In the list of upregulated genes, we sought enriched GO terms and functional annotations using the DAVID method (http://david.abcc.ncifcrf.gov) (Huang et al. 2007). Consistently with known effects of Pi deprivation, terms corresponding to phosphatase (P-value = $1.8.10^{-8}$), phosphate ion transport (P-value = $3.8.10^{-10}$), glycerophospholipid catabolic process (P-value = $3.3.10^{-3}$), galactolipid biosynthetic process (P-value = $1.5.10^{-5}$), phosphatidylcholine 1-acylhydrolase activity (P-value = $8.1.10^{-2}$), cell wall biogenesis/degradation (P-value = $2.6.10^{-1}$) or transcription regulation (P-value = $5.4.10^{-1}$) were enriched. The term corresponding to cytochrome P450 proteins was also enriched (P-value = $8.1.10^{-4}$) although we could not determine the precise pathway(s) in which these CYP proteins could be involved. In details, glycerolipids marker genes known to be upregulated in low Pi (Misson et al. 2005; Morcuende et al. 2007) were confirmed, i.e. *MGD2* (AT5G20410; Log2FC = 4.1), *MGD3* (AT2G11810; Log2FC = 6.6), *DGD1* (AT3G11670; Log2FC = 1.4), *DGD2* (AT4G00550; Log2FC = 1.8), *SQD2* (At5g01220; Log2FC = 4.3), *PLDζ2* (AT3G05630; Log2FC = 4.5) and *NPC4* (AT3G03530; Log2FC = 4.5) (Supplementary Table S3).

In shoots, 505 genes were down-regulated and 1,215 genes were up-regulated (Supplementary Table S4). We also sought enriched GO terms and functional annotations using the DAVID method in upregulated genes. As above, terms corresponding to acid phosphatase (P-value = $3.9.10^{-10}$), protein phosphatase 2C (P-value = $5.5.10^{-1}$), phosphate ion transport (P-value = 2.10^{-6}), cellular phosphate ion homeostasis (P-value = $7.1.10^{-7}$), glycerophospholipid catabolic process (P-value = $6.2.10^{-3}$), galactolipid biosynthetic process (P-value = $1.1.10^{-3}$), P-type ATPase (P-value = $2.8.10^{-1}$), cell wall modification (P-value = $8.6.10^{-1}$) or transcription regulation (P-value = $5.2.10^{-1}$) were enriched, confirming past analyses. By contrast with roots, additional terms corresponding to oxylipin biosynthetic process (P-value = $9.7.10^{-2}$), together with cytochrome P450 (P-value = $3.2.10^{-2}$) and secondary metabolites biosynthesis, transport, and catabolism (P-value = $7.2.10^{-2}$) were also identified. Glycerolipids marker genes upregulated in low Pi (Misson et al. 2005; Morcuende et al. 2007) were also confirmed, i.e. *MGD2* (Log2FC = 4.6), *MGD3* (Log2FC = 8.6), *DGD2* (Log2FC = 1.3), *SQD2* (Log2FC = 4.7), *PLDζ2* (Log2FC = 5.4) and *NPC4* (Log2FC = 5.7) (Supplementary Table S4). Transcriptional changes in shoots appeared therefore to involve oxylipins and, overall the magnitude of upregulation of marker genes was higher compared with roots.

Overall, this comparison of Arabidopsis grown in Pi-supplemented vs. Pi-deprived conditions validates the study dataset. The enrichment of GO terms corresponding to oxylipins in the comparison performed with green tissues supports therefore the possible activation of Jasmonic acid biosynthesis pathway reported earlier (Khan et al. 2016), at least in shoots.

We then focused our analysis on the expression of genes involved for JA biosynthesis (*AOS*, *AOC1*, *AOC2*, *AOC3*, *LOX2*, *LOX4*, *OPR3*, *JAR1*, *ACS1*, *ACS2*, *ACX1*, *AIM1*, *MFP2*, *KAT2*, *KAT5* and *JMT*), JA signaling (*FT AP2/ERF*, *JAZ1* to *JAZ10*, *COI1*), and as a control, glycerolipid remodeling (*NPC4*, *NPC5*, *PLDζ1*, *PLDζ2*, *MGD1*, *MGD2*, *MGD3*, *DGD1*, *DGD2*). Differential expression was measured in roots and shoots, 0.5 h after medium transfer, and highlights the same marker genes as those detected after the non-parametric comparison using all gene expression levels measured 0.5, 1 and 3 hours following medium transfer (Table 1). Concerning JA synthesis and signaling, *AOS* (in shoots), *AOC1* (in both roots and shoots), *AOC2* and *AOC3* (in shoots), *LOX2* (in roots and to some extent in shoots), *LOX4* (about two-fold increase in both roots and shoots), *JAZ5* (in roots) and *JAZ7* (in shoots) appeared upregulated in low Pi condition. Some modulated responses could therefore be observed between roots and shoots, but in both cases, genes involved in JA biosynthesis and signaling were significantly upregulated in response to Pi deprivation. Interestingly about half of the selected genes coding for JA biosynthesis or signaling components contained in their promoter region, one or more Pi-deprivation responsive elements (P1BS box 1, a known PHR1 binding element (Rubio et al. 2001)) (Table 1).

This comparison supports the fact that subsets of genes involved in JA biosynthesis might be co-regulated with genes involved in glycerolipid remodeling in response to Pi availability. We wondered whether these genes could be tuned down back to control level upon addition of Pi. The design of our experiment allowed investigating whether genes could be finely tuned following such resupply. To determine if these genes returned to basal expression levels after resupply differentially expressed genes and GO enrichment following Pi-resupply were determined, comparing the expression levels after 0.5, 1 and 3 hours in shoots. To that purpose, a partition of differentially expressed genes was performed using a *K*-mean method, with the number of partitions set to 10 (Dolch et al. 2017; Liu et al. 2014a). Each cluster consisted of genes with similar expression patterns (i.e. expression curves after 0.5, 1 and 3 hours) following Pi-resupply, with representative nearest mean curves, serving as prototypes for the clusters (shown in Fig. 2). Three clusters comprise genes upregulated following resupply, i.e. Cluster 2 consisting of genes with a strong and regular expression increase (Log2FC > 2), Cluster 4 with genes mainly upregulated 3 h following Pi-resupply and Cluster 5 with genes highly upregulated 1 h following Pi-resupply. Three clusters comprise genes downregulated following Pi-resupply, i.e. Cluster 7 consisting of genes with moderate but significant expression decline, Cluster 8 with genes mainly downregulated after 1 h resupply, and Cluster 10, a very large cluster containing genes with a regular expression decrease. This later cluster comprises genes with moderate to strong variation levels (Log2FC < -2). Based on GO term enrichment using two independent methods, GOseq (P-value < 5.10^{-2}) and DAVID (P-value $\leq 1.10^{-2}$), we analyzed these clusters to detect possible biological processes (BP) or molecular functions (MF) with a correlated dynamics of gene expression following Pi-resupply (Fig. 2). GO terms enriched in Cluster 10 highlight genes involved in the maintenance and repair of photosynthetic machinery, chloroplast division, cell mitosis and leaf morphogenesis, illustrating that the developmental reorientation in shoots does not occur within a short period following Pi resupply, but should rather require a longer time to return back to their level in control conditions.

GO terms corresponding to BP Jasmonic acid mediated signaling pathway, BP Regulation of jasmonic acid mediated signaling or BP Response to jasmonic acid were found enriched in Clusters 2, 4 and 5, *i.e.* showing therefore an upregulation upon Pi-resupply, whereas GO terms corresponding to BP Phosphate ion homeostasis, BP Phosphorus metabolic process, BP Lipid storage, BP Sulfolipid biosynthetic process, BP Galactolipid metabolic process, MF 1,2-diacylglycerol 3-beta-galactosyltransferase activity (i.e. synthesis of MGDG) were mainly found enriched in Cluster 8, showing a restoration of control expression level for these Pi-responsive genes (Fig. 2).

This analysis highlights that although genes involved in glycerolipid remodeling and JA metabolism or signaling are upregulated when plants are deprived of Pi, they appear to have uncoupled patterns of

expression following Pi-resupply. We addressed therefore the following questions: could JA signaling be involved in the dynamics and/or magnitude of the glycerolipid remodeling triggered by Pi-deprivation? Could JA signaling be involved in a reverse glycerolipid remodeling, returning back to initial state following Pi-resuply?

Jasmonic acid biosynthesis and signaling and glycerolipid remodeling are coupled following Pi deprivation and uncoupled following Pi resupply

The expression of some representative genes was examined via qRT-PCR (Fig. 3, white bars) in the leaves of wild type Col-0 Arabidopsis grown under different Pi regimen.

- When comparing Pi-supplemented and Pi-deprived conditions, genes involved in JA biosynthesis were upregulated (*LOX2*, *AOC1*, *AOC2*) or unchanged (*AOS*). The selected reporter gene for JA signaling, *JAZ10*, was consistently upregulated in Pi-deprived condition. As expected, all representative genes involved in glycerolipid reprogramming were upregulated (*NPC4*, *PLDζ2*, *MGD2*, *MGD3*, *DGD1*, *DGD2*, *SQD2*). The expression of *MGD1* increased slightly.
- Upon Pi-resupply, genes involved in glycerolipid reprogramming were tuned down. Only *MGD1* expression was enhanced. In contrast, all genes involved in JA biosynthesis and response were upregulated by the refeeding with Pi.

Previous reports have clearly demonstrated the production of JA and JA-Ile in *Arabidopsis* deprived of Pi, with different levels in roots and shoots (Khan et al. 2016). We sought whether the regulation of JA biosynthesis genes in the various Pi-regimen analyzed here, was also correlated with changes in JA levels in planta. To that purpose, we used an *Arabidopsis* transgenic line expressing the β-glucuronidase (GUS) reporter gene, fused with *JAZ1* under the cauliflower mosaic virus 35S promoter, p35S::JAZ1-GUS (Thines et al. 2007). Based on the interaction of JA-Ile with COI1, a component of the Skp1/Cullin/F-box SCF^{COI1} ubiquitin E3 ligase complex (Feys et al. 1994; Xie et al. 1998), JAZ1 is degraded. In this reporter system, an *in vivo* production of JA ad JA-Ile is therefore detected by a loss of GUS staining. The p35S::JAZ1-GUS transgenic line was cultivated under various phosphate regimen and the histochemical analysis was consistent with a low JA content in both roots and shoots in Pi-supplemented condition (Fig. 4, A), a production of JA mainly localized in the stem and leaves in Pi-deprived condition (Fig. 4, B) and no apparent decrease in JA content following Pi-resupply in leaves (Fig. 4, C). *In planta*, the level of JA appears therefore consistent with the variations of JA biosynthesis and response genes (Fig. 3, white bars).

We also sought whether the very rapid down-regulation of genes involved in glycerolipid remodeling (within hours following Pi-resupply) had an effect on a restoration of the control lipidomic profile. Using wild type samples collected in parallel, we extracted and analyzed glycerolipids 3 hours following

a resupply in Pi (Fig. 5, Col 0). We compared the profiles with that obtained in Pi-supplemented, used as a control. In photosynthetic tissues, plants grown in Pi-deprived medium exhibited the expected increase in SQDG, concomitant with a decrease in PG, consistent with the well-established SQDG-to-PG replacement in thylakoid membranes. In shoots, MGDG level increased slightly, DGDG level nearly doubled, whereas that of phospholipids and particularly PC decreased, consistently with the DGDG-to-PC exchange triggered by Pi deprivation. In roots, although lipid contents where much lower and more technically challenging to quantify, variations of SQDG, PG and MGDG were not observed in these non-green tissues, decrease in phospholipids were not statistically significant, whereas an increase of DGDG was clearly induced in Pi-deprived condition. In both roots and shoots, within a day following Pi-resupply, no significant change could be observed. Contrary to the down tuning of genes involved in glycerolipid remodeling (Fig. 3), these analyses did not support any glycerolipid remodeling back to control level rapidly after a resupply in Pi.

Taken together, these analyses show that JA biosynthesis and signaling genes are upregulated in Pi-deprived condition compared to Pi-supplemented condition, however, following Pi-resupply, expression of these genes is even more activated. Jasmonic acid presence in Pi-deprived condition and following a resupply in Pi is confirmed *in planta*. It is likely that JA and JA-derivatives accumulated upon Pi deprivation might maintain an upregulation of JA biosynthesis genes even though Pi signaling is attenuated, following the well-known positive feedback of the JA pathway, namely JA-induced JA biosynthesis (Sasaki et al. 2001). Only MGD1 expression followed the trend of JA biosynthesis and signaling genes, suggesting that this gene might be related to this interplay between JA signaling and responses to Pi variations.

Comparison of the gene expression reprogramming and the lipid remodeling in the WT and coi1 genetic backgrounds.

To confirm the link between JA signaling and glycerolipid remodeling induced by Pi variations, we considered a genetic background impaired in JA signaling. In previous works, the expression of the low-Pi responsive *MGD3* gene had been analyzed in the *pho1-7 aos* and *pho1-7 coi1-34* double mutants: its expression was significantly reduced compared to the *pho1-7* parent, suggesting that JA might control lipid remodeling. However, the increase of SQDG, MGDG and DGDG relatively to phospholipids was unchanged in *pho1-7 aos* and *pho1-7 coi1-34* double mutants compared to *pho1-7* (Khan et al. 2016). Here, we used a *coi1-16* mutant, impaired at the level of the COI1 dependent JA perception, subjected to various Pi regimen, to consider all possible Pi-responsive pathways. The *coi1-16* background is also known to contain a mutation in the *PEN2* gene (Westphal et al. 2008), a glycosyl hydrolase involved in the synthesis of antifungal glucosinolates (Bednarek et al. 2009). The *coi1-16*

mutant was previously used to unravel the interplay between JA and the response to potassium variations, with no effect which could be attributed to the *pen2* mutation (Armengaud et al. 2010). No biotic stress was exerted in the present study and only genes related to the *COI1* role and lipidome remodeling were examined. We compared gene expression and glycerolipid profiles in WT (Col-0) plants and *coi1-16* lines (Fig. 3, black bars and Fig. 5).

In the *coi1-16* mutant, the expression of most genes involved in JA biosynthesis or signaling was consistently reduced compared to the wild type, and was unaltered by variations in Pi, besides a very low upregulation observed for *JAZ1* or *JAZ10*. Interestingly, the expression of *AOC1* follows a pattern of a Pi-responsive gene in the *coi1-16* background (Fig. 3, black bars) although no P1BS box could be detected in its promoter region (Table 1). This suggests that expression of *AOC1* is controlled in response to Pi-availability by an unknown pathway and that JA masks this control by enhancing *AOC1* expression.

Genes involved in Pi-dependent glycerolipid remodeling are differentially expressed in the *coi1-16* background (Fig. 3, black bars). *PLDζ2* and *MGD2* show little changes. The overall expression of *NPC4*, *MGD3* and *DGD1* and to some extent of *DGD2* and *SQD2* is increased compared to WT, in particular in Pi-deprived condition, suggesting a negative control by JA. *MGD1* seems to show a slightly opposite response to Pi variations in *coi1-16* compared to Col-0, with a downregulated expression in Pi-deprived condition, unchanged after Pi-resupply (Fig. 3). However, this slight decrease observed in multiple analyses was not statistically significant based on a Dunett's test. *MGD3* expression is significantly activated in low-Pi in both WT and *coi1-16*, with a magnitude of upregulation moderated by JA. By contrast with previous analyses in the *pho1-7 coi1-34* double mutants and *pho1-7* parent (Khan et al. 2016), data shown in Fig. 3 suggest that the basal level of *MGD3* expression is increased in the *coi1-16* mutant when compared to Col-0. Although we have no clear explanation, this apparent discrepancy might be due to the *pho1-7* mutation, impairing phosphate assimilation constitutively. In this previous work, the decreased expression in *MGD3* was apparently contradictory with the observed increase in galactolipids (Khan et al. 2016); here the level of expression of *MGD3* (together with an increased level of PA, a known activator of MGD1, see below) is consistent with the higher level of MGDG in the *coi1-16* mutant. Altogether, these results suggest that JA exerts a sophisticated tuning of the expression of genes involved in glycerolipid homeostasis, regulating genes controlling phospholipid homeostasis in the endomembrane system (*NPC4*) and galactolipid metabolism in chloroplasts (*MGD3*, *DGD1*, and to some extent *MGD1*, *DGD2* and *SQD2*).

We sought whether these results were consistent with previous analyses of transcriptome change in Arabidopsis subjected to JA. In a recent comprehensive study, a whole-genome transcriptional

expression analysis was performed based on RNA extracted from leaves over 14 consecutive time points within 16 h following application of methyl JA (Hickman et al. 2017). Expression patterns of differentially expressed genes were clustered in 27 groups, 1 to 14 exhibiting an increased expression whereas 15 to 27, a downregulation. In this dataset, *MGD1* belonged to cluster 8 (consistent with the tendency observed in Fig. 3) and *MGD3* to cluster 16 (supporting the higher expression level we observed in the *coi1-16* mutant). This confirms the results reported on Figure 3 with a balanced control of MGDG synthesizing genes by JA, via opposite effects on MGD1 and MGD3.

When analyzing glycerolipids in the *coi1-16* mutant exposed to various Pi regimen, the most striking differences are observed in leaves at the levels of MGDG, DGDG, PC and phosphatidic acid (PA) (Fig. 5, A). Firstly, the basal level of PC appears lower in *coi1-16* and responds to Pi-deprivation like the WT. PC is the sole lipid showing a strong modification at its fatty acid composition level (Fig. 6). In this analysis, diacyls are expressed as the sum of carbon contained in the two fatty acid and the number of double bonds they harbor. PC-34 corresponds mainly to PC containing one fatty acid with 16 carbons and one fatty acid with 18 carbons and PC-36 corresponds to PC containing two 18-carbon fatty acids. In the *coi1-16*, PC-34-2 relative proportion increases, whereas that of PC-36-3 and PC-36-4 decrease, suggesting that molecular species enriched in fatty acids with 18 carbons are responsible for the global decrease in PC. Secondly, PA level is significantly higher in the *coi1-16* background. For this lipid intermediate, no change in fatty acid content is detected and no variation is observed in response to Pi. These data do not provide any evidence for the origin of this pool of PA, should it derive from a sub-pool or PC or another source. The subcellular distribution of this PA pool and its generation machinery being unknown, we could not speculate on the mechanism of its production in response to JA. It is known that PA can reach the chloroplast and activate galactolipid synthesis (Benning 2009; Botella et al. 2016; Dubots et al. 2012). Thirdly, and consistently with a high PA level, MGDG content appears higher than in the wild type, and remains unchanged regardless of Pi variations. DGDG level is twice as high in *coi1-16* as that in Col-0, and is even more increased in response to Pi-deprivation. The fatty acyl profiles in MGDG and DGDG showed little changes, except a slight increase in 18:3/18:3 in DGDG in roots (from 56.4% of total DGDG fatty acyls in Col-0 to 64.5% in *coi16-1*), reflecting an important contribution of MGD1 in most conditions in the synthesis of the bulk of galactolipids and a visible impact of the action of MGD3 in root non-photosynthetic plastids. It has been previously reported that even when *MGD3* was overexpressed, basal level of MGDG could show no change (Murakawa et al. 2014). Here, the increase in MGDG in the *coi16-1* background appears therefore most importantly due to MGD1 activation by PA.

Altogether these results highlight that more mechanisms are involved, rather than just a regulation by gene transcription, in the complex modulation of lipid homeostasis by JA. Interestingly, all mechanisms dissected here converge toward a control of galactolipid level.

Conclusion

We confirmed previous reports showing that JA biosynthesis and signaling are activated in *Arabidopsis* photosynthetic tissues when plants are cultivated in Pi-deprived conditions. Given the well-known role of JA in response to insects, this discovery was initially evaluated as a possible link between Pi deficiency and an enhanced herbivory resistance. JA could however act on other cellular responses, as shown here. In leaves, activation of JA coincides with a glycerolipid remodeling triggered by the lack of Pi. Concerning a possible action of JA on glycerolipid remodeling, our analysis of the *coi1-16* mutant is consistent with a control of the basal levels of MGDG, which is higher in this mutant possibly via an increased production of PA (an activator of MGDG synthesis) and by an increased expression of MGD3. This supports a role of JA in lowering MGDG production, at least by inhibiting *MGD3* expression. By contrast, upon Pi-deprivation, a role of JA in MGDG increase seems to occur, possibly by a moderate stimulation of the expression of *MGD1*, which needs to be confirmed in the future. When adding Pi to deprived plants, whereas glycerolipid remodeling genes are tuned back to normal, JA biosynthesis and signaling genes are even more activated. Our study shows that in this apparently reverse condition, JA also plays a role, and is therefore uncoupled from the low-Pi response. As a preliminary investigation of mechanisms involved, the effect of JA on two MGDG synthesis enzymes, *i.e.* a control of the activation of MGD1 (via an increase in PA) and a repression of MGD3 expression, might contribute to a fine tuning of MGDG variations in either Pi-deprivation or Pi-resupply. The existence of this balanced control of MGDG synthesis is supported by the complete loss of MGDG tuning in response to Pi variations in the *coi1-16* and is consistent with previous whole gene expression analyses. JA interplay with plant response to Pi variations is therefore complex, highlighting a fine tuning of the magnitude of gene expression levels in response to Pi. In the future, important questions need to be addressed, including the understanding of the lag between the rapid restoration of glycerolipid remodeling gene expression following Pi-resupply and the actual restoration of the glycerolipid profile; the elucidation of the mechanisms involved in the control of glycerolipid homeostasis by JA; and the evaluation of a possible involvement of JA in other glycerolipid remodeling processes occurring in response to other abiotic and biotic stresses and in relation with other hormonal controls.

Material and Methods.

Plant material and growth conditions.

All *Arabidopsis thaliana* lines used in this study were in the Col-0 ecotype. The *coi1-16* mutant and p35S::JAZ1-GUS (Thines et al. 2007) transgenic lines were provided by Dr Anthony Champion (IRD Montpellier). For all experiments plants were grown on modified Murashige and Skoog (MS) medium (Arnaud et al. 2014) supplemented with 2 µM $FeCl_2$ and either 500 µM KH_2PO4 (High Pi or "HPi") or 200 µM KCl (Low Pi or "LPi"). For the analysis of Pi starvation and resupply, seeds were cultivated in LPi conditions for 7 days and then transferred for 30 min, 1 hour or 3 hours either on LPi (LPi-LPi; "Pi-deprived") or HPi (LPi-HPi; "Pi-resupply"). The untreated control for these experiments was performed by growing plants on HPi conditions for 7 days before transferring them onto fresh HPi medium conditions during the appropriate control time (HPi-HPi; "Pi-supplemented"). Cultivations were performed on solid or liquid medium, as indicated (Kanno et al. 2016). For cultivation on agar plates, Low Pi Plant Agar (SIGMA A1296) was added to the supplemented MS medium at 0.8%. Seedlings were stratified in darkness (4°C, 24 hours), and grown in a 16-h-light/8-h-dark photoperiod at 21-24°C, in white light in vertical plates. For experiments using GUS reporters, seedlings were grown in liquid culture (24 wells falcon sterile plates, 3 mL MS medium per well, 10-15 seeds per well). Transfers were performed by moving directly the seedlings into the new medium. For lipids analyses and transcript level measurements, seedlings were germinated on stripes of Sefar Nitex 100 µM (30 - 40 seeds per stripe) placed into square plates of solid agar medium, and grown vertically. Transfers were performed by moving the Nitex stripes on new medium.

RNA extraction and RT-qPCR analyses

At least 30 individual seedlings were harvested per condition and stored in liquid nitrogen. A minimum of three biological replicates were collected per genotype/treatment. RNA was then isolated using the RNeasy Plant Mini Kit (Qiagen), following the manufacturer's instructions. Possible traces of DNA were eliminated with the RNase-Free DNase Set (Qiagen). 1 µg of RNA was used to obtain complementary DNA (cDNA) with the QuantiTect Reverse Transcription Kit (Quiagen). The quantitative polymerase chain reactions (qPCR) were carried out using SsoAdvanced™ Universal SYBR® Green Supermix (Biorad) and Biorad CFX96 Real-time PCR Detection System, according to the manufacturer's instructions. Three technical replicates were analyzed for each biological replicate. The TUB2 gene (AT1G75785) was used as reference. Primer pairs (forward, fwd and reverse, rev) used for amplification were the following: MGD1 (AT4G31780) fwd, TGGTTCGAGTCTCCGTAGGT and rev, CAAAGCTCTCCGGAACCACT; MGD2 (AT5G20410) fwd, ACAAGAAATTGGCATCTGCAT and rev, TGGTCCAGCTTTTGTGATGA; MGD3 (AT2G11810) fwd, AGAGGCCGGTTTAATGGAGT and rev, CATCAGAGGATGCACGCTAA; DGD1 (AT3G11670) fwd, ACGGTGAAGATGCAGTCGAG and rev, CCACAAACTTCCCCATGGCT; DGD2 (AT4G00550) fwd, ACGCTGACTCGCTATTTCACAAC and rev, TTTCCCATCGCCAAGGCTTCTG; SQD2 (At5G01220) fwd, TACCTGAAGCTCGGATTGCT and rev, TGTGAGAGTTCATCGCCTTG; PLDζ2

(AT3G05630) fwd, TCACGACAAGCAAGAACAGGTTAG and rev, AGTGCAGAGGAAGAGCACCATC; NPC4 (AT3G03530) fwd, TCCAAACCCGGGTCATCCTA and rev, GTTCATAACCGCGGAGGACA; AOC1 (AT3G25760) fwd, ACTCCTACTCGAGCTCTCTCTCAG and rev, GTTCTTGAACTTTGCTTGGTCTGG; AOC2 (AT3G25770) fwd, ACTGGAGCCTAGCGGAGTTA and rev, ACACAGCGATACGAGAAACAT; AOS (AT5G42650) fwd, GGTGGCGAGGTTGTTTGTGATTG and rev, TTCCTAACGGCGACGTACCAAC; LOX2 (AT2G18790) fwd, CAAGGATGCTGGCCTCTTAC and rev, TCGTCTCGTAACCATGAAAATC; LOX4 (AT1G72520) fwd, GGAAGACCACATCATCGGTCAAC and rev, AAACGGTTCGTCTCTAACGCTTG; JAZ1 (AT1G19180) fwd, AGCTTCACTTCACCGGTTCTTGGA and rev, TCTTGTCTTGAAGCAACGTCGTCA; JAZ10 (AT5G13220) fwd, TCGCAAGGAGAAAGTCACTGCAAC and rev, CGATTTAGCAACGACGAAGAAGGC; TUB2 (AT5G62690) fwd, GAGCCTTACAACGCTACTCTGTCTGTC and rev, ACACCAGACATAGTAGCAGAAATCAAG.

RNA-seq analyses

Roots and shoots of Arabidopsis grown under various Pi regimen were carefully cut and collected using a razor blade and RNA was extracted as described above. RNA quality and integrity were determined using the Nanodrop 1000 Spectrophotometer and Agilent Bioanalyser. Only high-quality RNA samples ($Abs_{260/280\ nm}$ ratios of 2.0–2.1) were used for RNA-seq library generation with the Illumina Truseq Stranded Total RNA sample prep kit. RNA-seq libraries were multiplexed and loaded per lane into the Illumina HiSeq flow cell v3. All sequencing protocols were carried out as per the manufacter's instructions using the Illumina HiSeq 1000 and HiSeq control software. RNA-seq reads were analysed using the Cufflinks package (Trapnell et al. 2012), version 2.1.1 and mapped onto the Arabidopsis TAIR10 genome (Kim et al. 2015) with the TAIR10 transcriptome annotation, using the DESeq2 method (Love et al. 2014; Varet et al. 2016). Clustering was achieved based on expression profiles as described previously (Dolch et al. 2017). In brief, partition of differentially expressed genes was performed using a K-mean method, with a number of partitions set to 6 and a clustering based on a Euclidian distance (Liu et al. 2014a). For each group we sought whether gene ontology (GO) terms could be enriched, either by the DAVID method (http://david.abcc.ncifcrf.gov) (Huang et al. 2007), using the corresponding Refseq gene IDs and with a default p-value threshold of 0.1, or using the GOseq R package (Young et al. 2010) with an identical p-value threshold. Based on GO enriched terms, a focused analysis of acyl-lipid and oxylipin pathways was performed. Illumina reads of all samples have been submitted to the Sequence Read Archive at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/sra) under accession number SRP133280.

Lipid extraction and analyses.

Roots and shoots of Arabidopsis grown under various Pi regimen were carefully cut and collected using a razor blade and a minimum of 100 µg fresh weight were stored in liquid nitrogen (3 biological replicates). Samples were lyophilized and lipids were extracted by the Folch method (Folch et al. 1957). Total glycerolipids were quantified from their fatty acids: in an aliquot fraction of extracted lipids, a known quantity of 15:0 was added and the fatty acids were converted into methyl esters (FAME) by a 1 hour incubation in 3 mL 2.5% H_2SO_4 in pure methanol at 100°C (Jouhet et al. 2003). The reaction was stopped by addition of 3 mL water and 3 mL hexane. The hexane phase was analyzed by a gas chromatography-flame ionization detector (GC-FID) (Perkin Elmer) on a BPX70 (SGE) column. FAME were identified by comparison of their retention times with those of standards (Sigma) and quantified by the surface peak method using 15:0 for calibration. For quantification of lipid classes by high-performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), fractions of extracted lipids corresponding to 25 nmol were suspended in 100 µL of chloroform/methanol 2:1, (v/v) containing 125 pmol of internal standards and analyzed as previously described (Jouhet et al. 2017). Internal standards were either obtained from Avanti Polar Lipids Inc. (for diacylglycerol, DAG 18:0-22:6; phosphatidylcholine, PC 18:0-18:0; phosphatidylethanolamine, PE 18:0-18:0; phosphatidylinositol, PI 18:0-18:0; phosphatidylserine, PS 18:0-18:0; phosphatidylglycerol, PG 18:0-18:0; phosphatidic acid, PA 18:0-18:0 and diphophatidylglycerol, DPG 14:0-14:0-14:0-14:0), synthesized by D. Lafont (Amara et al. 2010; Amara et al. 2009) (for galactolipids, MGDG 18:0-18:0 and DGDG 16:0-16:0) or purified from spinach thylakoid (Deme et al. 2014) and hydrogenated (Buseman et al;2006) (for sulfoquinovosyldiacylglycerol, SQDG 16:0-18:0). The HPLC separation method was adapted from (Rainteau et al. 2012). Lipid classes were separated using an Agilent 1200 HPLC system using a 150 mm×3 mm (length × internal diameter) 5 µm diol column (Macherey-Nagel), at 40°C. The mobile phases consisted of hexane/isopropanol/water/ammonium acetate 1 M, pH 5.3 [625/350/24/1, (v/v/v/v)] (A) and isopropanol/water/ammonium acetate 1M, pH 5.3 [850/149/1, (v/v/v)] (B). The injection volume was 20 µL. After 5 min, the percentage of B was increased linearly from 0% to 100% in 30 min and stayed at 100% for 15 min. This elution sequence was followed by a return to 100% A in 5 min and an equilibration for 20 min with 100% A before the next injection, leading to a total runtime of 70 min. The flow rate of the mobile phase was 200 µL/min. The distinct glycerolipid classes were eluted successively as a function of the polar head group. Mass spectrometric analysis was done on a 6460 triple quadrupole mass spectrometer (Agilent) equipped with a Jet stream electrospray ion source under following settings: drying gas heater, 260°C; drying gas flow 13 $L.min^{-1}$; sheath gas heater, 300°C; sheath gas flow; 11 $L.min^{-1}$; nebulizer pressure, 25 psi; capillary voltage, ± 5000 V; nozzle voltage, ± 1000. Nitrogen was used as collision gas. The quadrupoles Q1 and Q3 were operated at widest and unit resolution respectively. PC analysis was carried out in positive ion mode by scanning for precursors of m/z 184 at collision energy (CE) of 34 eV. SQDG analysis was carried out

in negative ion mode by scanning for precursors of m/z -225 at a CE of -56eV. PE, PI, PS, PG, PA, MGDG and DGDG measurements were performed in positive ion mode by scanning for neutral losses of 141 Da, 277 Da, 185 Da, 189 Da, 115 Da, 179 Da and 341 Da at CEs of 20 eV, 12 eV, 20 eV, 16 eV, 16 eV, 8 eV and 8 eV, respectively. Quantification was done by multiple reaction monitoring (MRM) with 30 ms dwell time. DAG and TAG species were identified and quantified by MRM as singly charged ions [M+NH4]+ at a CE of 16 and 22 eV respectively with 30 ms dwell time. DPG species were quantified by MRM as singly charged ions $[M-H]^-$ at a CE of -45 eV with 50 ms dwell time. Mass spectra were processed by MassHunter Workstation software (Agilent) for identification and quantification of lipids. Lipid amounts (pmol) were corrected for response differences between internal standards and endogenous lipids and by comparison with a quality control (QC). QC extract corresponds to an Arabidopsis lipid extract previously qualified and quantified by thin layer chromatography and gas chromatography coupled to ion flame detection (Jouhet et al. 2017).

GUS activity analyzes.

An *Arabidopsis* transgenic line expressing the β-glucuronidase (GUS) reporter gene, fused with *JAZ1* under the cauliflower mosaic virus 35S promoter, p35S::JAZ1-GUS (Thines et al. 2007) was cultivated under various phosphate regimen. Seedlings were prefixed, immediately after collection in ice-cold 90% acetone for 20 min on ice, then rinsed with cold water for 5 min, vacuum infiltrated for 10 min on ice with staining solution (50 mM sodium phosphate buffer pH 7.0, 0.2% Triton-X-100, 10 mM potassium ferrocyanide, 10 mM potassium ferricyanide, 1 mM X-Gluc) and incubated at 37°C in the dark for the indicated time. Samples were then cleared by progressive dehydration through ethanol series up to 100% and progressively re-hydrated prior to observation. Imaging was then performed using an Olympus SZX12 binocular microscope (with WHS-10X magnification system) equipped with a Nikon DXM1200C digital camera.

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References.

Acosta, I.F., Gasperini, D., Chetelat, A., Stolz, S., Santuari, L. and Farmer, E.E. (2013) Role of NINJA in root jasmonate signaling. *Proc Natl Acad Sci U S A* 110: 15473-15478.
Amara, S., Barouh, N., Lecomte, J., Lafont, D., Robert, S., Villeneuve, P., et al. (2010) Lipolysis of natural long chain and synthetic medium chain galactolipids by pancreatic lipase-related protein 2. *Biochim Biophys Acta* 1801: 508-516.
Amara, S., Lafont, D., Fiorentino, B., Boullanger, P., Carriere, F. and De Caro, A. (2009) Continuous measurement of galactolipid hydrolysis by pancreatic lipolytic enzymes using the pH-stat technique and a medium chain monogalactosyl diglyceride as substrate. *Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids* 1791: 983-990.
Andersson, M.X., Larsson, K.E., Tjellstrom, H., Liljenberg, C. and Sandelius, A.S. (2005) The plasma membrane and the tonoplast as major targets for phospholipid- to-glycolipid replacement and stimulation of phospholipases in the plasma membrane. *Journal of Biological Chemistry* 280: 27578-27586.
Andersson, M.X., Stridh, M.H., Larsson, K.E., Lijenberg, C. and Sandelius, A.S. (2003) Phosphate-deficient oat replaces a major portion of the plasma membrane phospholipids with the galactolipid digalactosyldiacylglycerol. *Febs Letters* 537: 128-132.
Andreou, A., Brodhun, F. and Feussner, I. (2009) Biosynthesis of oxylipins in non-mammals. *Progress in Lipid Research* 48: 148-170.
Armengaud, P., Breitling, R. and Amtmann, A. (2010) Coronatine-insensitive 1 (COI1) mediates transcriptional responses of Arabidopsis thaliana to external potassium supply. *Molecular plant* 3: 390-405.
Arnaud, C., Clement, M., Thibaud, M.C., Javot, H., Chiarenza, S., Delannoy, E., et al. (2014) Identification of phosphatin, a drug alleviating phosphate starvation responses in Arabidopsis. *Plant Physiol* 166: 1479-1491.
Awai, K., Marechal, E., Block, M.A., Brun, D., Masuda, T., Shimada, H., et al. (2001) Two types of MGDG synthase genes, found widely in both 16:3 and 18:3 plants, differentially mediate galactolipid syntheses in photosynthetic and nonphotosynthetic tissues in Arabidopsis thaliana. *Proc Natl Acad Sci U S A* 98: 10960-10965.
Bednarek, P., Pislewska-Bednarek, M., Svatos, A., Schneider, B., Doubsky, J., Mansurova, M., et al. (2009) A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. *Science* 323: 101-106.
Benning, C. (2009) Mechanisms of lipid transport involved in organelle biogenesis in plant cells. *Annu Rev Cell Dev Biol* 25: 71-91.
Botella, C., Sautron, E., Boudiere, L., Michaud, M., Dubots, E., Yamaryo-Botte, Y., et al. (2016) ALA10, a Phospholipid Flippase, Controls FAD2/FAD3 Desaturation of Phosphatidylcholine in the ER and Affects Chloroplast Lipid Composition in Arabidopsis thaliana. *Plant Physiol* 170: 1300-1314.
Boudiere, L., Michaud, M., Petroutsos, D., Rebeille, F., Falconet, D., Bastien, O., et al. (2014) Glycerolipids in photosynthesis: composition, synthesis and trafficking. *Biochim Biophys Acta* 1837: 470-480.
Bustos, R., Castrillo, G., Linhares, F., Puga, M.I., Rubio, V., Perez-Perez, J., et al. (2010) A central regulatory system largely controls transcriptional activation and repression responses to phosphate starvation in Arabidopsis. *PLoS Genet* 6: e1001102.
Chiou, T.J. and Lin, S.I. (2011) Signaling network in sensing phosphate availability in plants. *Annu Rev Plant Biol* 62: 185-206.
Cruz-Ramirez, A., Oropeza-Aburto, A., Razo-Hernandez, F., Ramirez-Chavez, E. and Herrera-Estrella, L. (2006) Phospholipase DZ2 plays an important role in extraplastidic galactolipid biosynthesis and phosphate recycling in Arabidopsis roots. *Proceedings of the National Academy of Sciences of the United States of America* 103: 6765-6770.
Deme, B., Cataye, C., Block, M.A., Marechal, E. and Jouhet, J. (2014) Contribution of galactoglycerolipids to the 3-dimensional architecture of thylakoids. *FASEB J* 28: 3373-3383.

Dolch, L.J., Lupette, J., Tourcier, G., Bedhomme, M., Collin, S., Magneschi, L., et al. (2017) Nitric Oxide Mediates Nitrite-Sensing and Acclimation and Triggers a Remodeling of Lipids. *Plant Physiol* 175: 1407-1423.
Dormann, P. and Benning, C. (2002) Galactolipids rule in seed plants. *Trends in Plant Science* 7: 112-118.
Dubots, E., Botte, C., Boudiere, L., Yamaryo-Botte, Y., Jouhet, J., Marechal, E., et al. (2012) Role of phosphatidic acid in plant galactolipid synthesis. *Biochimie* 94: 86-93.
Essigmann, B., Guler, S., Narang, R.A., Linke, D. and Benning, C. (1998) Phosphate availability affects the thylakoid lipid composition and the expression of SQD1, a gene required for sulfolipid biosynthesis in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences of the United States of America* 95: 1950-1955.
Feys, B., Benedetti, C.E., Penfold, C.N. and Turner, J.G. (1994) Arabidopsis Mutants Selected for Resistance to the Phytotoxin Coronatine Are Male Sterile, Insensitive to Methyl Jasmonate, and Resistant to a Bacterial Pathogen. *Plant Cell* 6: 751-759.
Folch, J., Lees, M. and Sloane Stanley, G.H. (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226: 497-509.
Gaude, N., Nakamura, Y., Scheible, W.R., Ohta, H. and Dormann, P. (2008) Phospholipase C5 (NPC5) is involved in galactolipid accumulation during phosphate limitation in leaves of Arabidopsis. *Plant Journal* 56: 28-39.
Hamburger, D., Rezzonico, E., MacDonald-Comber Petetot, J., Somerville, C. and Poirier, Y. (2002) Identification and characterization of the Arabidopsis PHO1 gene involved in phosphate loading to the xylem. *Plant Cell* 14: 889-902.
Hammond, J.P., Bennett, M.J., Bowen, H.C., Broadley, M.R., Eastwood, D.C., May, S.T., et al. (2003) Changes in gene expression in Arabidopsis shoots during phosphate starvation and the potential for developing smart plants. *Plant Physiol* 132: 578-596.
Hartel, H. and Benning, C. (2000) Can digalactosyldiacylglycerol substitute for phosphatidylcholine upon phosphate deprivation in leaves and roots of Arabidopsis? *Biochemical Society Transactions* 28: 729-732.
Hickman, R., Van Verk, M.C., Van Dijken, A.J.H., Mendes, M.P., Vroegop-Vos, I.A., Caarls, L., et al. (2017) Architecture and Dynamics of the Jasmonic Acid Gene Regulatory Network. *Plant Cell* 29: 2086-2105.
Huang, D.W., Sherman, B.T., Tan, Q., Kir, J., Liu, D., Bryant, D., et al. (2007) DAVID Bioinformatics Resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. *Nucleic Acids Research* 35: W169-W175.
Huang, T.K., Han, C.L., Lin, S.I., Chen, Y.J., Tsai, Y.C., Chen, Y.R., et al. (2013) Identification of downstream components of ubiquitin-conjugating enzyme PHOSPHATE2 by quantitative membrane proteomics in Arabidopsis roots. *Plant Cell* 25: 4044-4060.
Jouhet, J., Lupette, J., Clerc, O., Magneschi, L., Bedhomme, M., Collin, S., et al. (2017) LC-MS/MS versus TLC plus GC methods: Consistency of glycerolipid and fatty acid profiles in microalgae and higher plant cells and effect of a nitrogen starvation. *PLOS ONE* 12: e0182423.
Jouhet, J., Marechal, E., Baldan, B., Bligny, R., Joyard, J. and Block, M.A. (2004) Phosphate deprivation induces transfer of DGDG galactolipid from chloroplast to mitochondria. *J Cell Biol* 167: 863-874.
Jouhet, J., Marechal, E., Bligny, R., Joyard, J. and Block, M.A. (2003) Transient increase of phosphatidylcholine in plant cells in response to phosphate deprivation. *FEBS Lett* 544: 63-68.
Jouhet, J., Marechal, E. and Block, M.A. (2007) Glycerolipid transfer for the building of membranes in plant cells. *Prog Lipid Res* 46: 37-55.
Kanno, S., Arrighi, J.F., Chiarenza, S., Bayle , V., Berthome, R., Peret, B., et al. (2016) A novel role for the root cap in phosphate uptake and homeostasis. *eLife*.
Khan, G.A., Vogiatzaki, E., Glauser, G. and Poirier, Y. (2016) Phosphate Deficiency Induces the Jasmonate Pathway and Enhances Resistance to Insect Herbivory. *Plant Physiol* 171: 632-644.

Kim, S., Kim, M.S., Kim, Y.M., Yeom, S.I., Cheong, K., Kim, K.T., et al. (2015) Integrative structural annotation of de novo RNA-Seq provides an accurate reference gene set of the enormous genome of the onion (Allium cepa L.). *DNA Res* 22: 19-27.
Lin, S.I., Chiang, S.F., Lin, W.Y., Chen, J.W., Tseng, C.Y., Wu, P.C., et al. (2008) Regulatory network of microRNA399 and PHO2 by systemic signaling. *Plant Physiol* 147: 732-746.
Lin, Y.T., Chen, L.J., Herrfurth, C., Feussner, I. and Li, H.M. (2016) Reduced Biosynthesis of Digalactosyldiacylglycerol, a Major Chloroplast Membrane Lipid, Leads to Oxylipin Overproduction and Phloem Cap Lignification in Arabidopsis. *Plant Cell* 28: 219-232.
Liu, T., Zhang, S., Chen, J., Jiang, K., Zhang, Q., Guo, K., et al. (2014a) The transcriptional profiling of glycogenes associated with hepatocellular carcinoma metastasis. *PLoS One* 9: e107941.
Liu, T.Y., Huang, T.K., Tseng, C.Y., Lai, Y.S., Lin, S.I., Lin, W.Y., et al. (2012) PHO2-dependent degradation of PHO1 modulates phosphate homeostasis in Arabidopsis. *Plant Cell* 24: 2168-2183.
Liu, T.Y., Lin, W.Y., Huang, T.K. and Chiou, T.J. (2014b) MicroRNA-mediated surveillance of phosphate transporters on the move. *Trends Plant Sci* 19: 647-655.
Love, M.I., Huber, W. and Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology* 15: 550.
Michaud, M., Gros, V., Tardif, M., Brugiere, S., Ferro, M., Prinz, W.A., et al. (2016) AtMic60 Is Involved in Plant Mitochondria Lipid Trafficking and Is Part of a Large Complex. *Curr Biol* 26: 627-639.
Misson, J., Raghothama, K.G., Jain, A., Jouhet, J., Block, M.A., Bligny, R., et al. (2005) A genome-wide transcriptional analysis using Arabidopsis thaliana Affymetrix gene chips determined plant responses to phosphate deprivation. *Proceedings of the National Academy of Sciences of the United States of America* 102: 11934-11939.
Misson, J., Thibaud, M.C., Bechtold, N., Raghothama, K. and Nussaume, L. (2004) Transcriptional regulation and functional properties of Arabidopsis Pht1;4, a high affinity transporter contributing greatly to phosphate uptake in phosphate deprived plants. *Plant Mol Biol* 55: 727-741.
Mitsukawa, N., Okumura, S., Shirano, Y., Sato, S., Kato, T., Harashima, S., et al. (1997) Overexpression of an Arabidopsis thaliana high-affinity phosphate transporter gene in tobacco cultured cells enhances cell growth under phosphate-limited conditions. *Proc Natl Acad Sci U S A* 94: 7098-7102.
Morcuende, R., Bari, R., Gibon, Y., Zheng, W.M., Pant, B.D., Blasing, O., et al. (2007) Genome-wide reprogramming of metabolism and regulatory networks of Arabidopsis in response to phosphorus. *Plant Cell and Environment* 30: 85-112.
Muchhal, U.S., Pardo, J.M. and Raghothama, K.G. (1997) Phosphate transporters from the higher plant Arabidopsis thaliana. *Proc Natl Acad Sci U S A* 93: 10519-10523.
Muller, R., Morant, M., Jarmer, H., Nilsson, L. and Nielsen, T.H. (2007) Genome-wide analysis of the Arabidopsis leaf transcriptome reveals interaction of phosphate and sugar metabolism. *Plant Physiol* 143: 156-171.
Murakawa, M., Shimojima, M., Shimomura, Y., Kobayashi, K., Awai, K. and Ohta, H. (2014) Monogalactosyldiacylglycerol synthesis in the outer envelope membrane of chloroplasts is required for enhanced growth under sucrose supplementation. *Frontiers in plant science* 5: 280.
Nakamura, Y., Awai, K., Masuda, T., Yoshioka, Y., Takamiya, K. and Ohta, H. (2005) A novel phosphatidylcholine-hydrolyzing phospholipase C induced by phosphate starvation in Arabidopsis. *J Biol Chem* 280: 7469-7476.
Nussaume, L., Kanno, S., Javot, H., Marin, E., Pochon, N., Ayadi, A., et al. (2011) Phosphate Import in Plants: Focus on the PHT1 Transporters. . *Frontiers in plant science* 2: 83.
Okumura, S., Mitsukawa, N., Shirano, Y. and Shibata, D. (1998) Phosphate transporter gene family of Arabidopsis thaliana. *DNA Res* 5: 261-269.
Pant, B.D., Buhtz, A., Kehr, J. and Scheible, W.R. (2008) MicroRNA399 is a long-distance signal for the regulation of plant phosphate homeostasis. *Plant J* 53: 731-738.
Pant, B.D., Burgos, A., Pant, P., Cuadros-Inostroza, A., Willmitzer, L. and Scheible, W.R. (2015) The transcription factor PHR1 regulates lipid remodeling and triacylglycerol accumulation in Arabidopsis thaliana during phosphorus starvation. *J Exp Bot* 66: 1907-1918.

Park, J.H., Halitschke, R., Kim, H.B., Baldwin, I.T., Feldmann, K.A. and Feyereisen, R. (2002) A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in Arabidopsis due to a block in jasmonic acid biosynthesis. *Plant J* 31: 1-12.
Peret, B., Clement, M., Nussaume, L. and Desnos, T. (2011) Root developmental adaptation to phosphate starvation: better safe than sorry. *Trends Plant Sci* 16: 442-450.
Plaxton, W.C. and Tran, H.T. (2011) Metabolic adaptations of phosphate-starved plants. *Plant Physiol* 156: 1006-1015.
Rainteau, D., Humbert, L., Delage, E., Vergnolle, C., Cantrel, C., Maubert, M.A., et al. (2012) Acyl chains of phospholipase D transphosphatidylation products in Arabidopsis cells: a study using multiple reaction monitoring mass spectrometry. *PLoS One* 7: e41985.
Rellan-Alvarez, R., Lobet, G. and Dinneny, J.R. (2016) Environmental Control of Root System Biology. *Annu Rev Plant Biol* 67: 619-642.
Rubio, V., Linhares, F., Solano, R., Martin, A.C., Iglesias, J., Leyva, A., et al. (2001) A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes & development* 15: 2122-2133.
Sasaki, Y., Asamizu, E., Shibata, D., Nakamura, Y., Kaneko, T., Awai, K., et al. (2001) Monitoring of methyl jasmonate-responsive genes in Arabidopsis by cDNA macroarray: self-activation of jasmonic acid biosynthesis and crosstalk with other phytohormone signaling pathways. *DNA Res* 8: 153-161.
Taki, N., Sasaki-Sekimoto, Y., Obayashi, T., Kikuta, A., Kobayashi, K., Ainai, T., et al. (2005) 12-oxo-phytodienoic acid triggers expression of a distinct set of genes and plays a role in wound-induced gene expression in Arabidopsis. *Plant Physiol* 139: 1268-1283.
Thibaud, M.C., Arrighi, J.F., Bayle, V., Chiarenza, S., Creff, A., Bustos, R., et al. (2010) Dissection of local and systemic transcriptional responses to phosphate starvation in Arabidopsis. *Plant J* 64: 775-789.
Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., et al. (2007) JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. *Nature* 448: 661-665.
Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., et al. (2012) Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nature protocols* 7: 562-578.
Varet, H., Brillet-Gueguen, L., Coppee, J.Y. and Dillies, M.A. (2016) SARTools: A DESeq2- and EdgeR-Based R Pipeline for Comprehensive Differential Analysis of RNA-Seq Data. *PLoS One* 11: e0157022.
Wasternack, C. and Hause, B. (2013) Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. *Ann Bot* 111: 1021-1058.
Westphal, L., Scheel, D. and Rosahl, S. (2008) The coi1-16 mutant harbors a second site mutation rendering PEN2 nonfunctional. *Plant Cell* 20: 824-826.
Woo, J., MacPherson, C.R., Liu, J., Wang, H., Kiba, T., Hannah, M.A., et al. (2012) The response and recovery of the Arabidopsis thaliana transcriptome to phosphate starvation. *BMC Plant Biol* 12: 62.
Xie, D.X., Feys, B.F., James, S., Nieto-Rostro, M. and Turner, J.G. (1998) COI1: an Arabidopsis gene required for jasmonate-regulated defense and fertility. *Science* 280: 1091-1094.
Yan, J., Li, H., Li, S., Yao, R., Deng, H., Xie, Q., et al. (2013) The Arabidopsis F-box protein CORONATINE INSENSITIVE1 is stabilized by SCFCOI1 and degraded via the 26S proteasome pathway. *Plant Cell* 25: 486-498.
Yan, J., Zhang, C., Gu, M., Bai, Z., Zhang, W., Qi, T., et al. (2009) The Arabidopsis CORONATINE INSENSITIVE1 protein is a jasmonate receptor. *Plant Cell* 21: 2220-2236.
Yang, X.J. and Finnegan, P.M. (2010) Regulation of phosphate starvation responses in higher plants. *Ann Bot* 105: 513-526.
Young, M.D., Wakefield, M.J., Smyth, G.K. and Oshlack, A. (2010) Gene ontology analysis for RNA-seq: accounting for selection bias. *Genome biology* 11: R14.
Yu, B., Xu, C.C. and Benning, C. (2002) Arabidopsis disrupted in SQD2 encoding sulfolipid synthase is impaired in phosphate-limited growth. *Proceedings of the National Academy of Sciences of the United States of America* 99: 5732-5737.

Zhang, Z., Liao, H. and Lucas, W.J. (2014) Molecular mechanisms underlying phosphate sensing, signaling, and adaptation in plants. *J Integr Plant Biol* 56: 192-220.
Zheng, X. and Moriyama, E.N. (2013) Comparative studies of differential gene calling using RNA-Seq data. *BMC Bioinformatics* 14 Suppl 13: S7.

Table 1. Differential expression of genes involved in jasmonic acid biosynthesis and signaling in Pi-starved versus Pi-replete conditions. In the "Pi-supplemented" conditions, plants were grown for 7 days on HPi medium and then transferred to a fresh HPi medium. In the "Pi-deprived" condition, plants were grown for 7 days on LPi medium and then transferred to a fresh LPi medium. After 7 days, plants were transferred on an identical medium and then collected for RNA extraction. Based on whole genome RNA seq data, the differential expression of selected genes was determined in both shoots and roots and expressed in Log2FC. Genes involved in gene remodeling triggered by Pi deprivations, known to be upregulated, are given as internal control.

				Pi-deprived vs. Pi-supplemented (0.5h)	
		Short description	P1BS box I	Shoots Log2FC	Roots Log2FC
JA biosynthesis					
AOS	AT5G42650	allene oxide synthase	0	2.03587	-0.300299
AOC1	AT3G25760	allene oxide cyclase 1	0	2.84213	2.56893
AOC2	AT3G25770	allene oxide cyclase 2	1	1.04551	0.550625
AOC3	AT3G25780	allene oxide cyclase 3	0	1.04178	-0.311482
LOX2	AT3G45140	lipoxygenase 2	1	0.940651	1.53607
LOX4	AT1G72520	lipoxygenase 4	2	0.958026	0.773372
OPR3	AT2G06050	oxophytodienoate-reductase 3	1	0.757211	0.30015
JAR1	AT2G46370	Auxin-responsive GH3 family protein	0	0.740317	0.0841236
ACS1	AT4G05160	AMP-dependent synthetase and ligase family protein	0	0.642895	0.145448
ACS2	AT5G63380	AMP-dependent synthetase and ligase family protein	0	0.420248	-0.330739
ACX1	AT4G16760	acyl-CoA oxidase 1	0	0.409011	0.4213446
AIM1	AT4G29010	enoyl-CoA hydratase/isomerase family	1	0.408754	0.221613
MFP2	AT3G06860	multifunctional protein 2	0	0.246093	0.366468
KAT2	AT2G33150	peroxisomal 3-ketoacyl-CoA thiolase 3	3	0.200535	0.201582
KAT5	AT5G48880	peroxisomal 3-keto-acyl-CoA thiolase 2	1	0.190344	0.440682
JMT	AT1G19640	jasmonic acid carboxyl methyltransferase	0	-0.465981	-0.171726
JA signaling					
FT AP2/ERF	AT3G50260	cooperatively regulated by ethylene and jasmonate 1	1	1.46763	-0.124066
JAZ1	AT1G19180	jasmonate-zim-domain protein 1	2	0.818662	-0.202647
JAZ2	AT1G74950	jasmonate-zim-domain protein 2	0	0.323852	-0.0255306
JAZ3	AT3G17860	jasmonate-zim-domain protein 3	2	0.726571	0.123497
JAZ4	AT1G48500	jasmonate-zim-domain protein 4	1	0.45042	0.196468
JAZ5	AT1G17380	jasmonate-zim-domain protein 5	0	1.00279	-0.515517
JAZ6	AT1G72450	jasmonate-zim-domain protein 6	1	0.353224	-0.181203
JAZ7	AT2G34600	jasmonate-zim-domain protein 7	0	0.314164	1.26975
JAZ8	AT1G30135	jasmonate-zim-domain protein 8	0	0.428381	0.465424
JAZ9	AT1G70700	jasmonate-zim-domain protein 9	2	-0.264564	-0.540653
JAZ10	AT5G13220	jasmonate-zim-domain protein 10	0	-0.160352	0.389158
COI1	AT2G39940	RNI-like superfamily protein	0	-0.19787	-0.168118
Glycerolipid remodelling					
NPC4	AT3G03530	non-specific phospholipase C4	3	6.29128	4.74817
NPC5	AT3G03540	non-specific phospholipase C5	1	Nd	Nd
PLDζ1	AT3G16785	phospholipase D zeta 1	0	0.204936	0.0101217
PLDζ2	AT3G05630	phospholipase D zeta 2	4	5.93978	4.34813
MGD1	AT4G31780	monogalactosyldiacylglycerol synthase 1	2	0.38767	0.695703
MGD2	AT5G20410	monogalactosyldiacylglycerol synthase 2	4	4.49902	4.39337
MGD3	AT2G11810	monogalactosyldiacylglycerol synthase 3	0	8.67635	6.48075
DGD1	AT3G11670	digalactosyldiacylglycerol synthase 1	1	0.718678	1.45735
DGD2	AT4G00550	digalactosyldiacylglycerol synthase 2	0	1.845	1.9015
SQD2	AT5G01220	sulfoquinovosyldiacylglycerol synthase	4	4.45593	4.45593

Figure Legends

Figure 1. Experimental design. Three phosphate growing conditions were compared. In the "Pi-supplemented" conditions, plants were grown for 7 days on HPi medium and then transferred to a fresh HPi medium. In the "Pi-deprived" condition, plants were grown for 7 days on LPi medium and then transferred to a fresh LPi medium. In the "Pi-resupply" condition, plants were grown for 7 days on LPi medium and then transferred to a fresh HPi. Plants were then collected for various analyses, *i.e.* 0.5, 1 and 3 hours after transfer. In some analyses, plants were collected 24 hours after transfer.

Figure 2. **K-mean clustering of gene expression profiles in Pi-deprived *Arabidopsis* following a resupply with phosphate.** Plants were grown for 7 days on LPi medium and then transferred to a fresh HPi, corresponding to the "Replenished" condition. Plants were then collected 0.5, 1 and 3 hours after transfer. RNA was extracted and gene expression determined as described in the Methods section. A partition of differentially expressed genes was performed using a K-mean method, with a number of partitions set to 10 and a clustering based on a Euclidian distance (Liu et al. 2014a). Each cluster consists of genes with similar expression profiles following Pi-resupply, with representative nearest mean curves shown, serving as prototypes. Three clusters comprise genes upregulated following Pi resupply (Clusters 2, 4 and 5), whereas three clusters comprise genes which expression is downregulated (Clusters 7, 8, 10). Based on gene ontology (GO) term enrichment using two independent methods, GOseq (P-value < 5.10^{-2}) and DAVID (P-value ≤ 1.10^{-2}), genes involved in enriched molecular function (MF) and biological processes (BP) related to phosphate incorporation and homeostasis, glycerolipid remodeling and jasmonic acid biosynthesis and signaling are indicated.

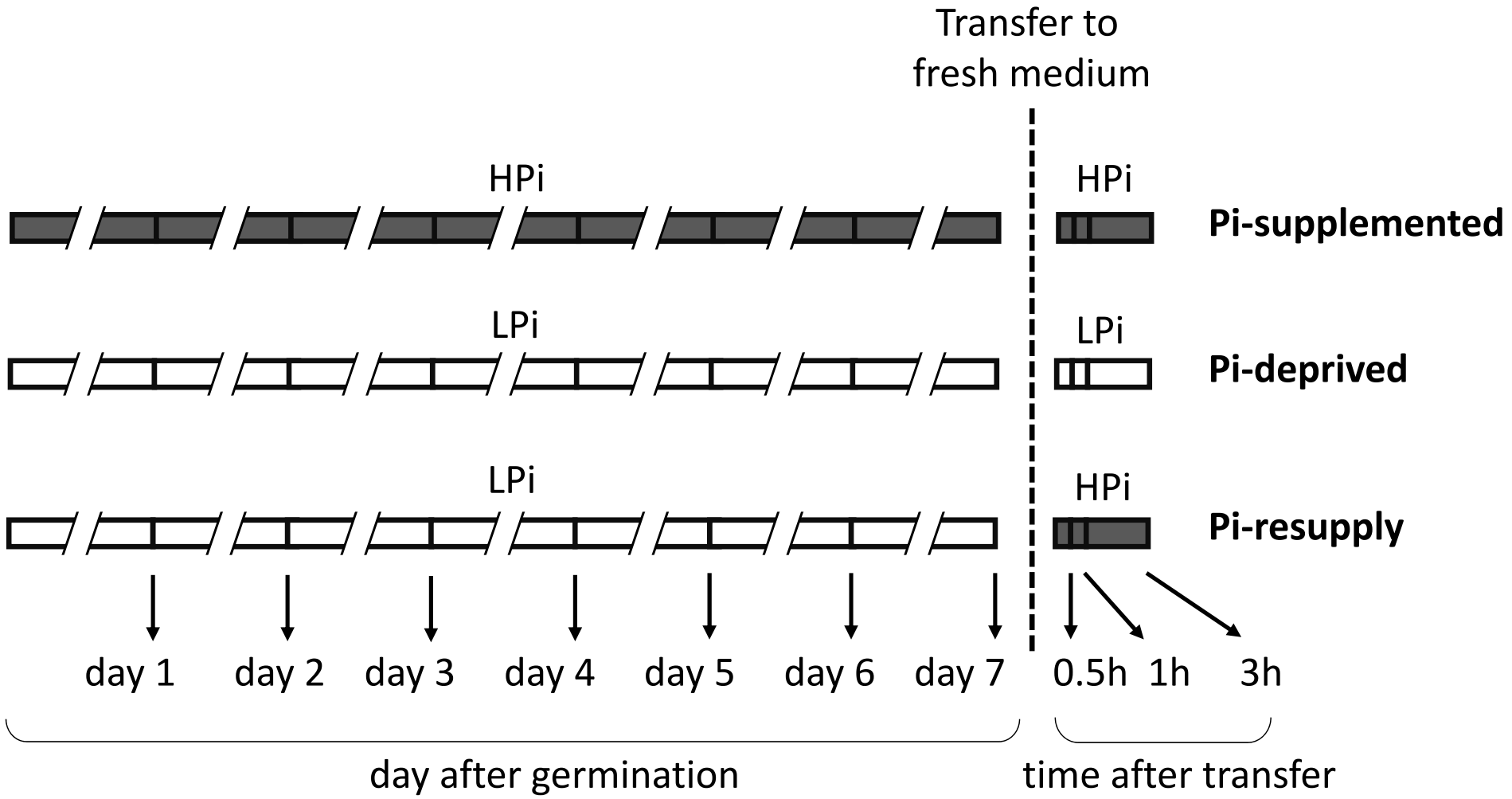
Figure 3. Expression of genes involved in JA biosynthesis and signaling and in lipid remodeling in the WT Col0 strain and in the *coi1-16* mutant of *Arabidopsis thaliana*. Plants were cultivated as described in Fig. 1. Leaves were carefully collected 3 hours after medium transfer, RNA were extracted and gene expression levels were evaluated by RT-qPCR. Data were normalized as described in Methods, using the expression of TUB2 as a reference. Experiments correspond to biological triplicates. Error bars show standard deviations. White bars, expression levels in Col0; solid bars, expression levels in *coi1-16*. Two-way ANOVA Dunett's test: (*) P-value < 10^{-1}; (**) P-value < 10^{-2}; (***) P-value < 10^{-3}; (****) P-value < 10^{-4}.

Figure 4. **Histochemical detection of the GUS activity in *Arabidopsis* 35S::JAZ1::GUS transgenic plants, submitted to different phosphate growing conditions. A, Pi-supplemented condition.** Plants were grown for 7 days on HPi medium and then transferred to a fresh HPi medium for 24 hours. Scale bar: 1mm. **B, Pi-deprived condition.** Plants were grown for 7 days on LPi medium and then transferred to a fresh LPi medium for 24 hours. Scale bar: 500 µm. **C, Pi-resupply condition.** Plants were grown for 7 days on LPi medium and then transferred to a fresh HPi medium for 24 hours. Scale bar: 500 µm.

Figure 5. Glycerolipid profiles in *Arabidopsis thaliana* Col-0 and coi-16 mutant treated in various phosphate regimen. A. Glycerolipid profiles in shoots. B. Glycerolipid profiles in roots. Plants were grown as shown in Fig. 1, *i.e.* 7 days on HPi medium and then transferred to a fresh HPi medium for 3 hours in Pi-supplemented condition (Pi-supplem.); 7 days on LPi medium and then transferred to a fresh LPi medium for 3 hours in Pi-deprived

condition and eventually 7 days on LPi medium and then transferred to a fresh HPi medium for 3 hours in Pi-resupply condition. Roots and shoots were carefully collected, and glycerolipids were extracted as described in Methods. Each lipid class is expressed in nmol per mg of dry weight (DW). Green bars, glycerolipid profiles in Col-0; brown bars, glycerolipid profiles in coi1-16. Data correspond to three independent biological replicates. Error bars correspond to standard deviation. DAG, diacylglycerol; DGDG, digalactosyldiacylglycerol; DPG, diphosphatidylglycerol; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; SQDG, sufoquinovosyldiacylglycerol, TAG, triacylglycerol. Significant differences between Col-0 and coi1-16 are indicated by a star (p-value < 0.05; two-tailed t-test).

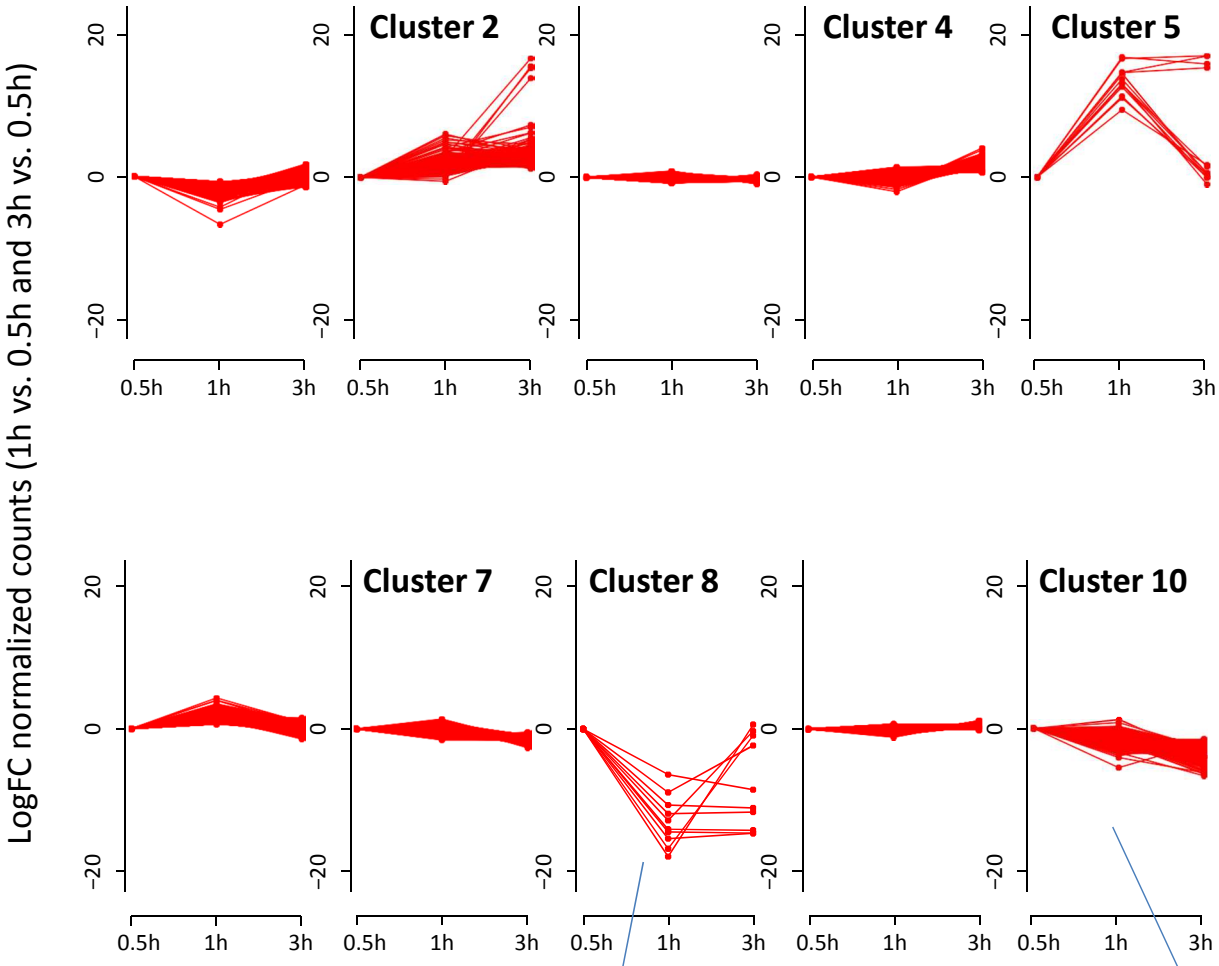
Figure 6. Leaf phosphatidylcholine diacyl profiles in *Arabidopsis thaliana* Col-0 and coi-16 mutant treated in various phosphate regimen. Plants were grown as shown in Fig. 1, *i.e.* 7 days on HPi medium and then transferred to a fresh HPi medium for 3 hours in Pi-supplemented condition (Pi-supplem.); 7 days on LPi medium and then transferred to a fresh LPi medium for 3 hours in Pi-deprived condition and eventually 7 days on LPi medium and then transferred to a fresh HPi medium for 3 hours in Pi-resupply condition. Shoots were carefully collected, and glycerolipids were extracted as described in Methods. Phosphatidylcholine (PC) diacyl moieties were analyzed as described in Methods. Data correspond to three independent biological replicates. Error bars correspond to standard deviation. Diacyls are expressed as the sum of carbon contained in the two fatty acids and the number of double bonds they harbor. For instance, PC containing a 16:0 and an 18:2 fatty acids is expressed as PC-34-2; PC containing two 18:2 fatty acids is expressed as PC-36-4. Green bars, PC profiles in Col-0; brown bars, PC profiles in coi1-16. Data correspond to three independent biological replicates. Significant differences between Col-0 and coi1-16 are indicated by a star (p-value < 0.05; two-tailed t-test).


Transfer to
fresh medium
HPi
HPi
Pi-supplemented
LPi
LPi
Pi-deprived
LPi
HPi
Pi-resupply
day 1
day 2
day 3
day 4
day 5
day 6
day 7
0.5h
1h
3h
day after germination
time after transfer

2729 genes; GO enrichment analysis using Goseq (P-value < 5.10^{-2})
GO term
BP Phosphate ion transmembrane transport
BP Galactolipid biosynthetic process
BP Endoplasmic reticulum to chloroplast transport
BP **Jasmonic acid** mediated signaling pathway
BP Regulation of **jasmonic acid** mediated signaling
BP Response to **jasmonic acid**

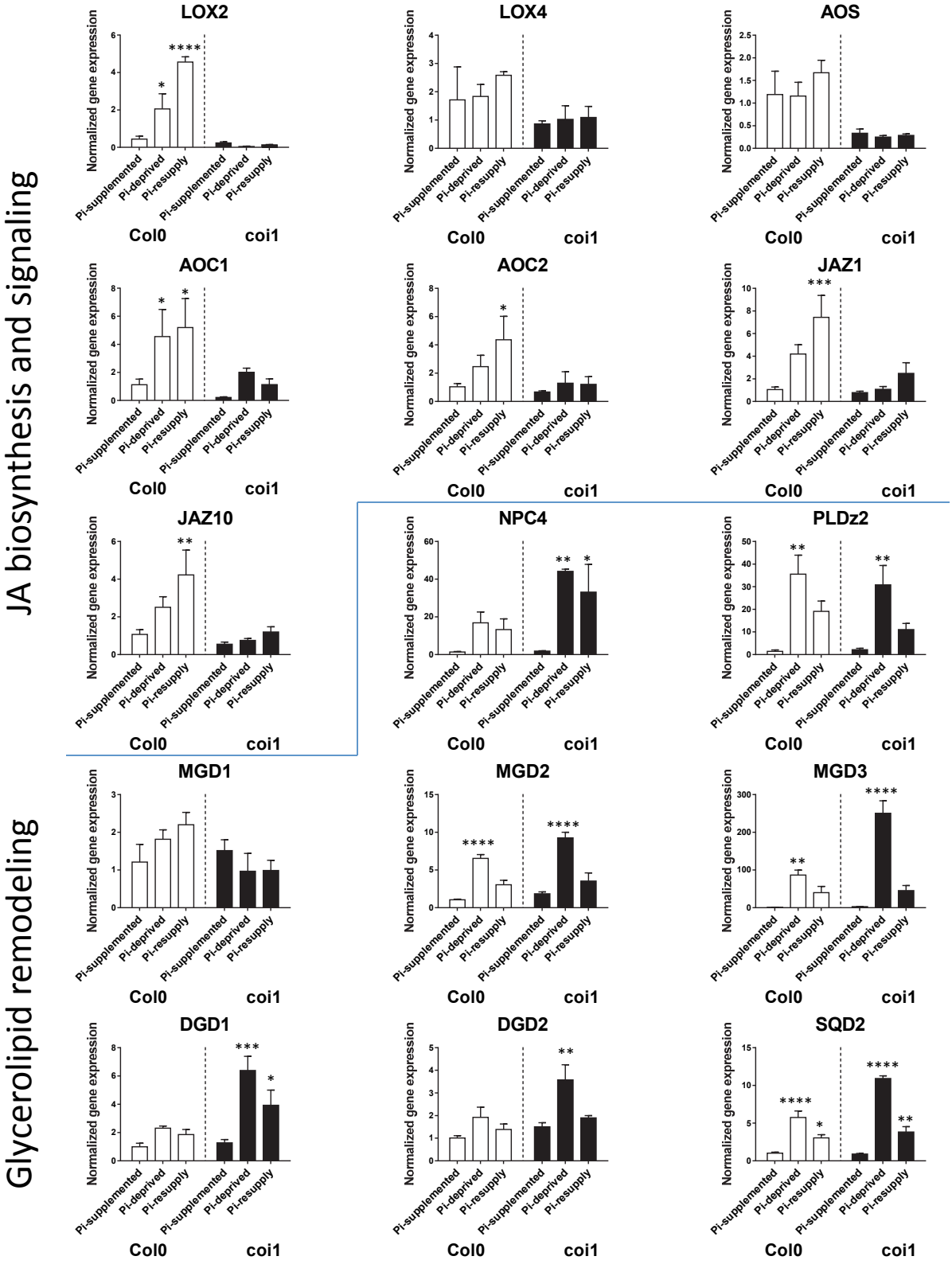
3482 genes; GO enrichment analysis using Goseq (P-value < 5.10^{-2})
GO term
BP Phosphate ion transmembrane transport
BP Galactolipid biosynthetic process
BP Fatty acid oxidation
BP **Jasmonic acid** biosynthetic process
BP Regulation of **jasmonic acid** mediated signaling
BP Response to **jasmonic acid**

1255 genes; GO enrichment analysis using Goseq (P-value < 5.10^{-2})
GO term
BP Phosphate ion transmembrane transport
BP Galactolipid biosynthetic process
BP CDP-diacylglycerol biosynthetic process
BP Response to **jasmonic acid**
MF **Jasmonoyl-isoleucine-hydroxylase** activity
BP Root epidermal cell differenciation

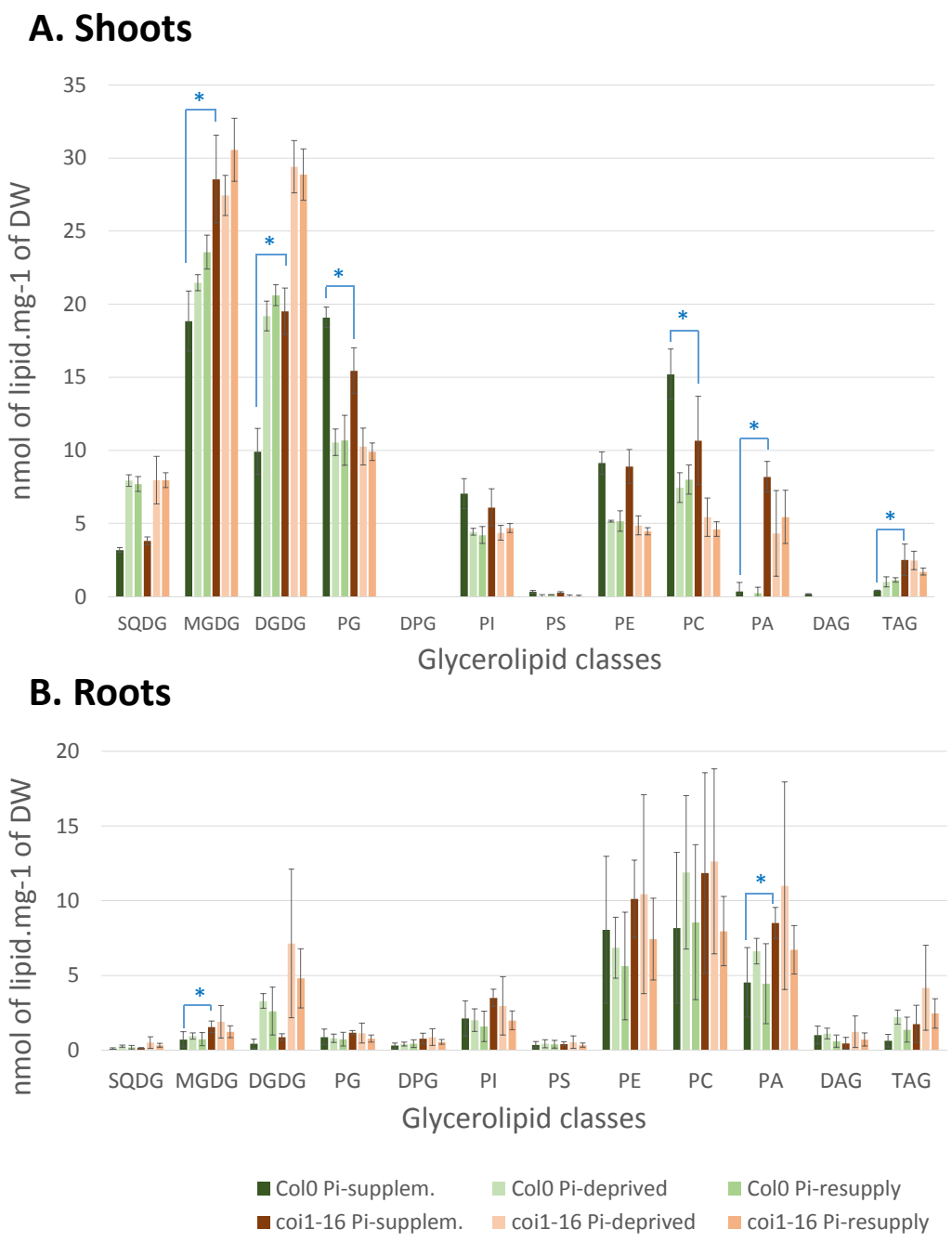


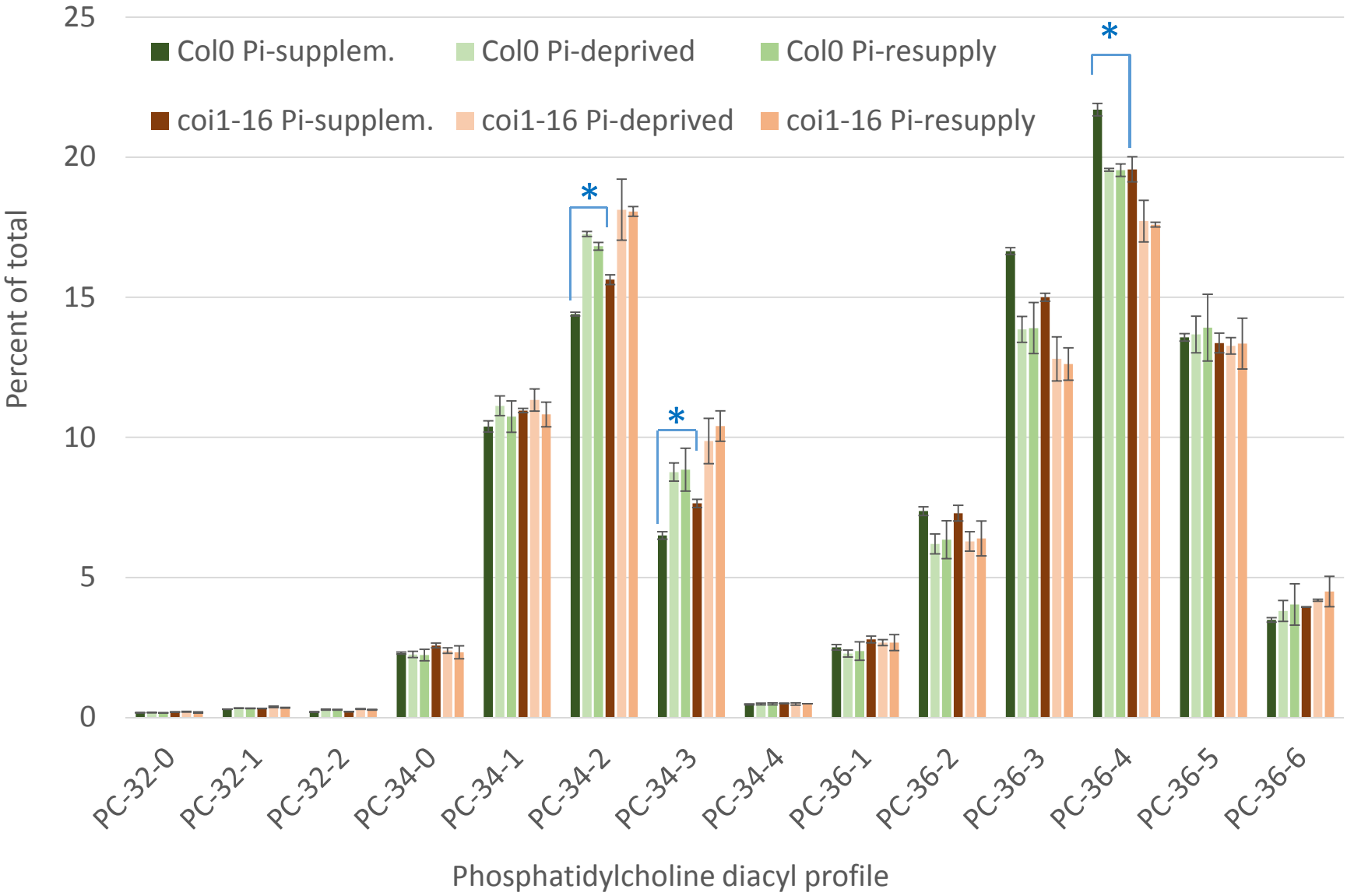
440 genes; GO enrichment analysis using Goseq (P-value < 5.10^{-2})
GO term
BP Phosphate ion homeostasis
BP Phosphorus metabolic process
BP Lipid storage
BP Sulfolipid biosynthetic process
BP Galactolipid metabolic process
MF 1,2-diacylglycerol 3-beta-galactosyltransferase activity
BP **Jasmonic acid** stimulus
BP **Jasmonic acid** mediated signaling pathway

5744 genes; GO enrichment analysis using Goseq (P-value < 5.10^{-2})
GO term
BP Photosynthesis
BP ATP synthesis coupled proton transport
BP photosystem I assembly
BP photosystem II assembly
BP photosystem II stabilization
BP photosystem II repair
BP mitotic nuclear division
BP chloroplast fission
BP leaf morphogenesis


JA biosynthesis and signaling
Glycerolipid remodeling
LOX2
LOX4
AOS
AOC1
AOC2
JAZ1
JAZ10
NPC4
PLDz2
MGD1
MGD2
MGD3
DGD1
DGD2
SQD2
Normalized gene expression
Pi-supplemented
Pi-deprived
Pi-resupply
Col0
coi1


A. Pi-supplemented
500um
B. Pi-deprived
500um
C. Pi-resupply
500um


A. Shoots
nmol of lipid.mg-1 of DW
35
30
25
20
15
10
5
0
SQDG MGDG DGDG PG DPG PI PS PE PC PA DAG TAG
Glycerolipid classes
B. Roots
nmol of lipid.mg-1 of DW
20
15
10
5
0
SQDG MGDG DGDG PG DPG PI PS PE PC PA DAG TAG
Glycerolipid classes
Col0 Pi-supplem.
Col0 Pi-deprived
Col0 Pi-resupply
coi1-16 Pi-supplem.
coi1-16 Pi-deprived
coi1-16 Pi-resupply


25
20
15
10
5
0
Percent of total
Col0 Pi-supplem.
Col0 Pi-deprived
Col0 Pi-resupply
coi1-16 Pi-supplem.
coi1-16 Pi-deprived
coi1-16 Pi-resupply
*
*
*
PC-32-0
PC-32-1
PC-32-2
PC-34-0
PC-34-1
PC-34-2
PC-34-3
PC-34-4
PC-36-1
PC-36-2
PC-36-3
PC-36-4
PC-36-5
PC-36-6
Phosphatidylcholine diacyl profile