



HAL
open science

Phosphatidic Acid in Plant Hormonal Signaling: From Target Proteins to Membrane Conformations

Yaroslav Kolesnikov, Serhii Kretynin, Yaroslava Bukhonska, Igor Pokotylo, Eric Ruelland, Jan Martinec, Volodymyr Kravets

► **To cite this version:**

Yaroslav Kolesnikov, Serhii Kretynin, Yaroslava Bukhonska, Igor Pokotylo, Eric Ruelland, et al.. Phosphatidic Acid in Plant Hormonal Signaling: From Target Proteins to Membrane Conformations. *International Journal of Molecular Sciences*, 2022, 23 (6), pp.3227. <10.3390/ijms23063227>. <hal-03659128>

HAL Id: hal-03659128

<https://hal.science/hal-03659128v1>

Submitted on 4 May 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization



Review

Phosphatidic Acid in Plant Hormonal Signaling: From Target Proteins to Membrane Conformations

Yaroslav Kolesnikov¹, Serhii Kretynin¹, Yaroslava Bukhonska¹, Igor Pokotylo¹, Eric Ruelland² , Jan Martinec^{3,*} and Volodymyr Kravets^{1,*}

¹ V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry National Academy of Sciences of Ukraine, Murmanska Street, 1, 02660 Kiev, Ukraine; kolesnikov@bpci.kiev.ua (Y.K.); sergey.bpci@gmail.com (S.K.); yasya.yaroslavka@gmail.com (Y.B.); pokotylo@bpci.kiev.ua (I.P.)

² Sorbonne Universités, Génie Enzymatique et Cellulaire, UMR CNRS 7025, Université de Technologie de Compiègne, CEDEX, 60203 Compiègne, France; eric.ruelland@u-pec.fr

³ Institute of Experimental Botany of the Czech Academy of Sciences, Rozvojová 263, 165 00 Prague, Czech Republic

* Correspondence: martinec@ueb.cas.cz (J.M.); kravets@nas.gov.ua (V.K.)

Abstract: Cells sense a variety of extracellular signals balancing their metabolism and physiology according to changing growth conditions. Plasma membranes are the outermost informational barriers that render cells sensitive to regulatory inputs. Membranes are composed of different types of lipids that play not only structural but also informational roles. Hormones and other regulators are sensed by specific receptors leading to the activation of lipid metabolizing enzymes. These enzymes generate lipid second messengers. Among them, phosphatidic acid (PA) is a well-known intracellular messenger that regulates various cellular processes. This lipid affects the functional properties of cell membranes and binds to specific target proteins leading to either genomic (affecting transcriptome) or non-genomic responses. The subsequent biochemical, cellular and physiological reactions regulate plant growth, development and stress tolerance. In the present review, we focus on primary (genome-independent) signaling events triggered by rapid PA accumulation in plant cells and describe the functional role of PA in mediating response to hormones and hormone-like regulators. The contributions of individual lipid signaling enzymes to the formation of PA by specific stimuli are also discussed. We provide an overview of the current state of knowledge and future perspectives needed to decipher the mode of action of PA in the regulation of cell functions.

Keywords: phospholipase; diacylglycerol kinase; phospholipid; phosphatidic acid; signal transduction; targets; biologically active substance; autophagy



Citation: Kolesnikov, Y.; Kretynin, S.; Bukhonska, Y.; Pokotylo, I.; Ruelland, E.; Martinec, J.; Kravets, V.

Phosphatidic Acid in Plant Hormonal Signaling: From Target Proteins to Membrane Conformations. *Int. J. Mol. Sci.* **2022**, *23*, 3227. <https://doi.org/10.3390/ijms23063227>

Academic Editors: Małgorzata Pietrowska-Borek and Sławomir Borek

Received: 16 December 2021

Accepted: 7 March 2022

Published: 17 March 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Phosphatidic acid (PA) is a minor membrane glycerolipid composed of glycerol-3-phosphate and two fatty acid chains. PA is an intermediate and regulator of glycerolipid metabolism in yeast, animals and plants [1–4]. Under basal conditions, PA is formed de novo in the endoplasmic reticulum and plastids mainly by acylation of glycerol-3-phosphate catalyzed by specific acyltransferases [5]. PA is also an important lipid second messenger in cells, acting in part through interaction with various effector proteins [6,7]. The level of PA, like that of most phospholipids in cells, is highly dynamic and changes specifically, rapidly (seconds to minutes) and transiently (i.e., eventually reverting to basal level) in response to various extracellular, hormonal or stress stimuli [8,9]. These changes are made possible by various phospholipases, lipid kinases and phosphatases. The generation of signaling PA occurs via the activation of phospholipase D (PLD), which cleaves structural membrane lipids (e.g., phosphatidylcholine) to generate PA and free headgroups (e.g., choline) [10–13] (Figure 1). Signaling PA can also be produced by the phosphorylation of diacylglycerol (DAG, Figure 1) in a reaction catalyzed by diacylglycerol kinase (DGK) [14–16]. The formation of DAG itself is the result of the activity of two classes of phospholipases

C: one that hydrolyzes phosphatidylinositol phosphates (phosphatidylinositol-specific phospholipase C, PI-PLC) or one that hydrolyzes structural lipids (phosphatidylcholine-specific or non-specific phospholipase C – PC-PLC or NPC) [17,18]. As a part of lipid turnover, PA can be dephosphorylated back into DAG by lipid phosphate phosphatases (LPP) [19,20].

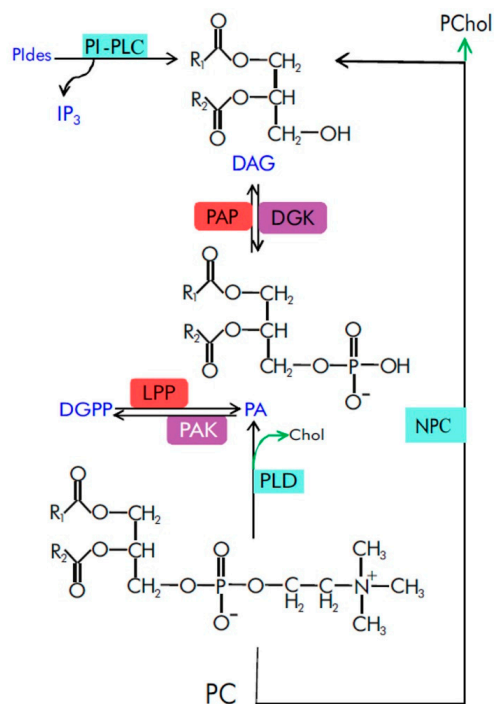


Figure 1. A diagram representing key enzymatic steps of PA production involved in cell signaling. Phosphatidylcholine (PC) is shown as the principal substrate for PLD-generated PA. Signaling lipid mediators and their active products are highlighted in dark blue. Their non-signaling by-products or lipid precursors are shown in black. Phospholipases involved in DAG and PA metabolism are shown in light blue rectangles. Lipid kinases that phosphorylate DAG and PA are shown in violet rectangles. Lipid phosphatases that dephosphorylate DGPP and PA are shown in light red rectangles. Black arrows indicate pathways that generate signaling lipids; green arrows indicate pathways that generate by-products. Note that no PAK-coding gene is currently identified in *Arabidopsis*. Chol, choline; DGK, diacylglycerol kinase; DGPP, diacylglycerol pyrophosphate; IP₃, inositol 1,4,5-trisphosphate; LPP, lipid phosphate phosphatase; NPC, nonspecific phospholipase C; PAK, phosphatidic acid kinase; PAP, phosphatidic acid phosphatase; PChol, phosphocholine; PC, phosphatidylcholine; Pides, phosphoinositides; PI-PLC, phosphatidylinositol-specific phospholipase C; PLD, phospholipase D; R₁, R₂, fatty acids.

Each class of enzymes involved in the production of PA is represented by multiple isoforms (isoenzymes) [4,21]. Regulation of their activity by extracellular agonists may represent a specific mechanism to control lipid signaling in cells [9,22]. Changes to activity or abundance of particular isoenzymes acting in PA production may be essential for stress tolerance [23,24] or growth/developmental responses [25–28].

In cell membranes, PA molecules are not compositionally uniform. They are represented by different molecule classes (molecular species) depending on the length and degree of desaturation of fatty acid moieties they incorporate [29]. Accordingly, PA produced in the course of cell signaling is also heterogeneous. *At*PLD α 1 activity stimulated by phytohormone abscisic acid (ABA) leads to 34:2-PA, 34:3-PA, 36:3-PA, 36:4-PA, 36:5-PA and 36:6-PA production [30]. The profile of PA molecules generated by *At*PLD δ is different: this enzyme additionally generates 34:1-PA and 36:2-PA, but not 36:3-PA and 36:6-PA [31]. Different PA molecular species can affect target proteins in a non-identical way leading to specific physiological consequences. Different PA molecular species bind to proteins acting

in different branches of ABA responses with dissimilar affinity: di18:1-PA has been shown to be more active in binding protein phosphatase 2C *AtABI1* than di16:0-PA, di18:0-PA and di18:2-PA [32]; 16:0/18:2-PA binds the NADPH oxidase subunit *AtRbohD* preferentially to 16:0/16:0-PA and 18:0/18:0-PA [30]; the binding of 18:1/18:1-PA, 16:0/18:1-PA and 16:0/18:2-PA to the sphingosine kinases *AtSPHK1* and *AtSPHK2* is higher than that of 16:0/16:0-PA [33].

The concentration of PA significantly differs depending on plant tissue type, plant developmental phase, treatment condition as well as registration technique. For example, in ³²P-labelled *Arabidopsis*, basal PA levels range from 2% (in germinating seeds and seedlings) to nearly 8% (in leaf peels) of all labeled phospholipids [25]. In the roots of soybean seedlings, the level of PA, as detected by MS, was found to constitute up to 2.5% of total cell lipids [34]. The level of PA in *Arabidopsis* seedlings detected by GC was found to constitute several mol% [35]. In rice leaves, the level of PA analyzed by mass spectrometry was observed to be in the range of 2.5 to 5 nmol/mg [36].

The general topology of PA signaling in plants seems to be similar to that of animals. Plasma membrane or intracellular receptors activate PA production by directly or indirectly affecting the activity of PA-producing enzymes; then, the formed PA binds target proteins and/or affects membrane conformation. However, some differences exist between PA signaling in animals and plants. The first important difference concerns the interrelationships between DAG and PA. For example, in animals, DAG has several protein targets in comparison with a huge number of PA targets and a broader array of signaling properties that are very distinct from those of DAG. On the other hand, in plants, no DAG targets were found, suggesting that PA is the lipid signal instead of DAG. Although conversion of DAG to PA by the DGK is the major route to terminate DAG signaling in animals, there are several examples indicating that DGKs modulate signaling events by producing PA [37]. The second difference is associated with enzymes of PA production. No NPC gene was found in animals in comparison to plants. Furthermore, the PLD family in plants is more heterogenic in comparison with classical PLDs (PLD1 and PLD2) in animals, and PLD substrate specificity is wider in plants than that in animals [38]. The third difference in PA signaling between animals and plants concerns the pathways of PA metabolic conversions. PA phosphatases from animals can generate DAG from PA produced by PLD, and this newly formed DAG possesses signaling and metabolic function [39]. However, such pathways in plants produce DAG for the synthesis of other lipids (e.g., triacylglycerols, galactolipids), but the role of the pathway in signaling was not discovered in plants. In addition, diacylglycerol pyrophosphate, a phosphorylated form of PA with signaling functions, has been found in plants, but not animals [40]. Such a multifaced role of PA in living organisms suggests an evolutionary significance of its signaling mechanisms.

2. Spatiotemporal Characteristics of Phosphatidic Acid as a Signaling Molecule

PA has several properties underlying its signaling functions. PA has a specific pattern of intracellular localization. Under basal conditions, PA has been detected in the plasma membrane (PM) in various plant objects: growing tobacco pollen tubes [41,42], mature trichomes [43], epidermal and meristematic root cells and shoot tissues [44]. However, PA has also been detected in cell membranes other than PM. PA has been detected in the nucleus of *Arabidopsis* epidermal root cells undergoing cell division [44] and in chloroplast membranes [45]. Interestingly, the level of nuclear PA in *Arabidopsis* seedlings has been shown to be affected by the activities of lipid signaling enzymes located at the PM, cytosol and endoplasmic reticulum [3]. The level of PA in the PM is highly dynamic even under basal conditions, possibly due to the combination of rapid lateral diffusion of PA and PLD, DGK and LPP activities leading to local accumulation of PA [41]. In response to extracellular stimuli (e.g., abscisic acid), rapid changes in PA signaling, specifically at the PM of plant cells, have been reported [46].

Another important property of PA is its high turnover rate. The rapid turnover of PA contributes to its low level in unstressed cells compared to structural phospholipids. The increase in PA content in response to a stimulus is usually transient [46].

Cells of different organs and tissue types have different capacities to respond to extracellular stimuli. At least in part, this could be due to differences in their basal PA levels. These levels vary depending on the growth stage of the plant [47], type of plant organ [48,49] or developmental phase of the organ [50]. This could explain why the stimulated PA levels were different in plant organs [51] and tissue types [46,52]. The differential capacity for PA signaling at different developmental stages can be illustrated by the high levels of DGK and PLD proteins in developing maize ears [53] and the high basal PA content in cotton fiber cells compared to ovule cells [54]. The expression levels of many PA signal transduction enzymes also differ depending on the plant developmental phase (Figure 2). These differences may thus determine the extent of activation of lipid signal transduction in response to extracellular (or endogenous) stimuli and control its regulatory performance.

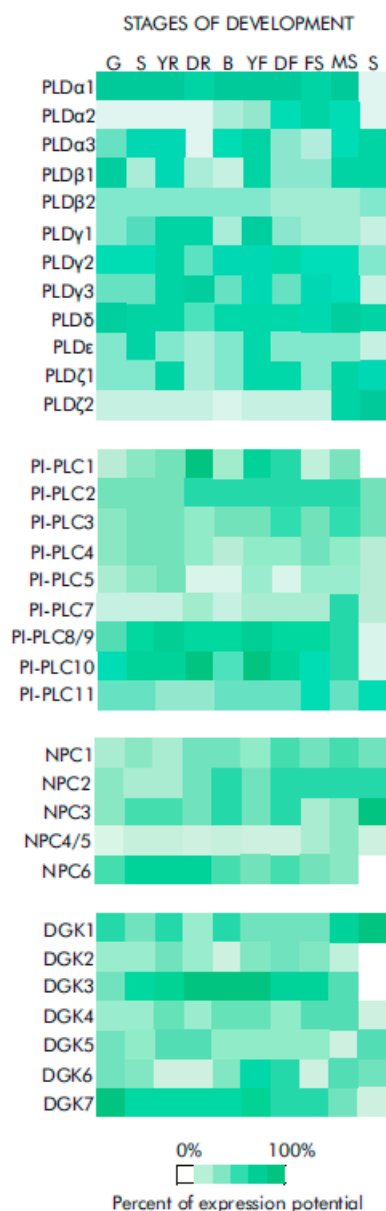


Figure 2. Expression levels of genes encoding enzymes of PA signaling at different developmental stages of *Arabidopsis thaliana* obtained using “Genevestigator” database [55]. Data were collected from

data based on classical array experiments ("AT_AFFY_ATH1" platform). The relative values of gene expression (percent of expression potential, log₂ scale) are shown. Percent of expression potential denotes the relative transcript abundance given as percentage of the level in the developmental stage where the gene is expressed at maximum level. Color saturation corresponds to the specific level of gene expression. White color signifies an absence of data. Number of samples: germinated seed (515), seedling (2781), young rosette (830), developed rosette (2219), bolting (358), young flower (720), developed flower (1003), flowers and siliques (274), mature siliques (93), senescence (18). Gene identifiers are as follows: PLD α 1 (At3g15730), PLD α 2 (At1g52570), PLD α 3 (At5g25370), PLD ϵ (At1g55180), PLD β 1 (At2g42010), PLD β 2 (At4g00240), PLD γ 1 (At4g11850), PLD γ 2 (At4g11830), PLD γ 3 (At4g11840), PLD δ (At4g35790), PLD ζ 1 (At3g16785), PLD ζ 2 (At3g05630); PI-PLC1 (At5g58670), PI-PLC2 (At3g08510), PI-PLC3 (At4g38530), PI-PLC4 (At5g58700), PI-PLC5 (At5g58690), PI-PLC6 (At2g40116), PI-PLC7 (At3g55940), PI-PLC8 (At3g47290), PI-PLC9 (At3g47220), PI-PLC10 (At4G34920), PI-PLC11 (At3G19310); NPC1 (At1g07230), NPC2 (At2g26870), NPC3 (At3g03520), NPC4 (At3g03530), NPC5 (At3g03540), NPC6 (At3g48610); DGK1 (At5g07920), DGK2 (At5g63770), DGK3 (At2g18730), DGK4 (At5g57690), DGK5 (At2g20900), DGK6 (At4g28130), DGK7 (At4g30340). Abbreviations of *Arabidopsis* developmental stages: G, germinated seed; S, seedling; YR, young rosette; DR, developed rosette; B, bolting; YF, young flower; DF, developed flower; FS, flowers and siliques; MS, mature siliques; S, senescence.

3. Effect of PA on Membrane Status

Signaling PA is produced rapidly, transiently and at low concentrations by different pathways in cellular membranes. PA regulatory influence on metabolism is mediated by interactions with target proteins and by inducing effects on membrane functions, charge and conformations.

Phospholipids and enzymes of phospholipid turnover are involved in the control of membrane trafficking [56,57] and the transduction of specific extracellular signals in plants [58,59]. It becomes clear that plant hormone-induced PA accumulation might also be associated with membrane trafficking pathways. This can be illustrated by the fact that *n*-butanol, an inhibitor of PA formation by PLD, impairs translocation of NON-EXPRESSION OF PATHOGENESIS-RELATED GENES 1 (NPR1) to the nucleus in response to salicylic acid [60].

Endocytosis and exocytosis induced by PA may be an important part of PA signaling in plants. For example, activation of *Brassica napus* PLD α 1 during compatible pollination has been hypothesized to promote exocytosis required for pollen germination through the production of PA in the PM [61]. This notion is also supported by genetic and pharmacological data suggesting that the production of PA modulates basal cellular membrane trafficking. Suppression of PA production by inhibition of PLD or DGK activity compromised membrane trafficking, disrupted the deposition of cell wall material (pectins) at the pollen tube tip and inhibited pollen tube growth [62]. Inhibition of PLD also blocked the membrane localization of clathrin and affected the localization of PIN2 (an auxin transporter) in root epidermal cells [63]. In *dgk4 Arabidopsis* plants, a reduced rate of membrane secretion and recycling, as well as inhibited pollen tube growth, were observed [64]. PA and lysophosphatidic acid acyltransferases (AtLPAAT4 and AtLPAAT5) that also generate PA were suggested to be critical for the secretory pathway involved in protein trafficking of the auxin transporter PIN2 and the aquaporin PIP2;7 [65].

A recent study on the functions of PLD δ -family phospholipase in tobacco showed that pollen tubes support a high level of expression of PLD δ genes during growth. For some of the PLD δ isoforms, the unique localization was detected, and the sequences important for binding to the PM were determined [42]. It has been noted that the increased level of PA, formed by the overexpression of *NtPLD δ 3* isoform, causes specific morphological phenotypes indicating a violation of the balance of vesicular traffic and membrane recirculation [42].

PA and the enzymes that produce it can affect membrane trafficking and endocytosis by interacting with their specific proteins. *AtPLD α 1* is associated with both microtubules and clathrin-coated vesicles [66], *AtECA2* and ANTH domain proteins that recruit clathrin to membranes to facilitate vesicle budding [67], and CHC (Clathrin Heavy Chain) proteins [68]. Other membrane trafficking events regulated by PA include PA-mediated membrane recruitment of *NtEXO70A1a* and *NtEXO70B1*—two proteins involved in targeting the vesicle tethering complex (exocyst) for exocytosis [69].

Some of the effects of PA on membrane conformations are local: the accumulation of PA leads to a negative spontaneous curvature that facilitates interactions with specific proteins. Indeed, the binding of the Epsin-like Clathrin Adaptor 1 (*AtECA1*) to PA was dependent on membrane curvature stress [70]. Hence, binding was achieved only after a certain threshold level of negative curvature stress. This suggests that the binding of *AtECA1* requires some degree of hydrophobic interaction with the membrane. PA-binding proteins may take advantage of the presence of bulky hydrophobic residues such as tryptophan to insert themselves into the membrane interface, having a negative spontaneous curvature [71].

Since PA is a component of cell membranes, it interacts not only with proteins but also with other membrane lipids. Studies performed on artificial membranes show that PA binds to phosphatidylcholine (via electrostatic interactions) [72] and stacks with other PA molecules (at low pH or in the presence of divalent cations) [73]. These interactions could be a factor that regulates the affinity of PA towards protein binding and membrane tethering.

PA signaling could be functionally linked to pH changes. The protonation state of PA has been found to depend on the local pH and to affect the efficiency of PA binding to its effector proteins [46].

4. Binding of Phosphatidic Acid to Target Proteins

A number of PA-binding proteins have now been identified. Many of them are thought to play a role in plant signal transduction. However, a detailed characterization of the biological role, as well as the deciphering of the interaction with PA at the molecular level, is available for only some of them (Table 1). Since no PA-recognition motif has yet been identified in plant proteins (as well as animal proteins), it has been proposed that complex structural folds of a protein are involved in PA recognition. PA-binding modules typically consist of an amphipathic α -helix with positively charged amino acids on one side (e.g., lysine-rich sequence motif) and hydrophobic residues on the other [74]. Similar structural folds have been found in proteins involved in plant hormone signaling, suggesting that this may indeed be a common mechanism of PA-protein interaction (Figure 3).

An interaction between a PA and a protein implicates specific amino acid residues of the latter. It has been shown that positively charged polybasic residues (Lys, Arg) [30,75] are involved in PA binding. The negative charges carried by anionic PA thus enable the recruitment of proteins with polybasic regions [44]. In addition, other types of amino acids (e.g., His in the defensin *HsAFP1* [76] and Pro in the lipid kinase *AtPIP1K1* [77]) are suggested to act in the binding of PA. Together, this enables the establishment of electrostatic and hydrophobic interactions as well as hydrogen bonds between PA and a target protein. His, Trp, Ser, Ala and Gly are also commonly present in PA-binding modules of proteins. This is, for example, true for CDeT11-24, a desiccation-related phosphoprotein of *Craterostigma plantagineum*. The Lys-rich motif of this protein is responsible for the interaction with the PA phosphomonoester moiety, while a region of nonpolar amino acids, seen in a helical wheel projection of the lysine-rich motif of CDeT11-24, may be involved in hydrophobic interactions with the acyl chain of PA [78]. His42 and His51 are thought to contribute to the pH sensitivity of the binding of PA to the Epsin-like Clathrin Adaptor 1 [71]. Non-basic amino acids could play a supporting role in PA binding and cause a change in the PA binding affinity of a protein—this could be an explanation for the differences in PA binding to the NADPH oxidases *AtRbohD* and *AtRbohF* [30].

Table 1. Plant PA-binding proteins with characterized mechanism of PA binding and identified role in cell signaling.

PA Target	PA-Binding Site	Effect on Activity	Role in Signaling	Lipid Signaling Pathway	Notes (PA Binding Specificity)	Refs.
<i>AtRbohD</i> (At5g47910), <i>AtRbohF</i> (At1g64060), respiratory burst oxidase homolog protein D and F	Arg149, Arg150, between EF-hands and N-terminus (in cytosolic region), in the vicinity of phosphorylation motif (<i>AtRbohD</i>); residues 105 to 341 (<i>AtRbohF</i>)	Stimulation at PM (<i>AtRbohD</i>)	ABA signaling, ROS generation, ROS-dependent NO production, stomatal closure	<i>AtPLDα1</i>	di18:1PA, di18:2PA, 16:0–18:1 PA, 16:0–18:2PA, 18:0–18:2 PA), but not to di16:0PA, di18:0PA; <i>AtRbohD</i> has higher affinity to PA than <i>AtRbohF</i>	[60]
<i>AtSPHK1</i> (At4g21540), <i>AtSPHK2</i> (At4g21534), sphingosine kinases	VSGDGI motif (<i>AtSPHK1</i>)	Stimulation by promoting substrate binding	ABA signaling, <i>AtPLDα1</i> activation (for amplification of ABA response), stomatal closure, seed germination, root elongation	<i>AtPLDα1</i>	Strong binding to 18:1/18:1PA, 16:0/18:1PA, 16:0/18:2PA	[71,72]
<i>AtRopGEF8</i> (At3g24620), guanine nucleotide exchange factor 8	Lys13, Lys18 in <i>AtGEF8</i> PRONE domain and N-terminus	Stimulation of <i>AtGEF8</i> activity towards small GTPase ROP7	ABA signaling activation		di18:2PA, di18:1PA, 16:0–18:1PA, 16:0–18:2PA, 16:0–18:1PA, 18:0–18:2PA	[61]
<i>AtRGS1</i> (At3g26090), regulator of G-protein signaling	Lys259 in RGS domain	Inhibition of GTPase-accelerating protein activity of <i>AtRGS1</i>	ABA signaling activation, inhibition of seed germination, root growth and stomatal responses	-	-	[73]
<i>AtMAP65-1</i> (At5g55230), a microtubule-associated protein	Lys and Arg from 53KRRK55 and 61KSR63 sequences, Ser-428 in 428SK429 sequence, out of microtubule binding or phosphorylation regions	Promotion of <i>AtMAP65-1</i> -induced microtubule-bundling activity	Salt stress signaling enhances tubulin polymerization involving <i>AtMAP65-1</i> increases salt tolerance by microtubule stabilization	<i>AtPLDα1</i>	16:0–18:1PA, 16:0–18:2PA, 18:0–18:1PA, 18:0–18:2PA, but not di18:0PA	[74]
<i>AtMPK6</i> (At2g43790), a mitogen-activated protein kinase 6	-	Stimulation	Salt stress signaling, salt tolerance by <i>AtMPK6</i> -dependent phosphorylation of <i>AtSOS1</i>	<i>AtPLDα1</i>	Significantly to di18:1PA, di18:2PA, 16:0–18:1PA, 16:0–18:2 PA, 18:0–18:2PA	[75]

Table 1. Cont.

PA Target	PA-Binding Site	Effect on Activity	Role in Signaling	Lipid Signaling Pathway	Notes (PA Binding Specificity)	Refs.
<i>AtMKK7</i> (At1g18350), <i>AtMKK9</i> (At1g73500), a mitogen-activated protein kinase kinases	-	Activation, translocation to PM	Salt stress signaling, salt tolerance, maximization of the signal transduction efficiency	<i>AtPLDα1</i>	Strongly to di18:1PA, 16:0–18:1PA, 16:0–18:2PA, 18:0–18:2PA	[76]
<i>AtMPK3</i> (At3g45640)/ <i>AtMPK6</i> (At2g43790), a mitogen-activated protein kinases	-	Stimulation, interaction with and phosphorylation of hypoxia-related transcription factor RAP2/12	Hypoxia stress signaling, translocation to nucleus and stimulation of hypoxia-related transcription factor RAP2/12, feedback inhibition of PA production, tolerance to submergence	<i>AtPLDα1</i> , <i>AtPLDδ</i>	-	[77]
<i>AtPINOID</i> (At2g34650), a protein kinase	Lys119 to Lys121	Stimulation, tethering to PM	Salt stress signaling, salt tolerance, enhanced <i>AtPINOID</i> -dependent <i>AtPIN2</i> phosphorylation and PM localization, activation of PIN2-mediated auxin efflux and redistribution	<i>AtPLDα1</i> , <i>AtPLDδ</i>	di18:1PA, di18:2PA, 16:0–18:1PA, 16:0–18:2PA, 18:0–18:1PA, 18:0–18:2PA	[65]
<i>AtSnRK2.4</i> (At1g10940)	-	Phosphorylation of SnRK2 targets, affects its interaction with PA and subcellular localization	-	-	16:0/18:1 PA	[78]
<i>OsAKT2</i> (Os05g35410), a potassium channel	Arg190/Lys191, Arg644/Arg645, Arg755/Arg756 in ANK domain, cytoplasmic domain, S4 voltage sensor	Inhibition	Seedlings growth under short-day conditions	-	Strongly to 16:0–18:2PA, much weaker to di16:0PA, di18:0PA, di18:1PA, di18:2PA, 16:0–18:1PA, 18:0–18:1PA, 18:0–18:2 PA	[67,79]
<i>OsGID1</i> (Os05g33730), a gibberellin receptor	Arg79, Arg82	Tethering to nucleus	Gibberellin signaling, promotion of <i>OsSLR1</i> (a suppressor DELLA protein) degradation	<i>OsPLDα6</i>		[80]

Abbreviations: ABA, abscisic acid, Arg, arginine (R), Lys, lysine (K), NO, nitric oxide, PM, plasma membrane, PP2C, protein phosphatase 2C, ROS, reactive oxygen species.

Interestingly, several PA-binding sites are sometimes located in different parts of a protein. It should be noted that multiple basic and hydrophobic amino acid residues have been shown to be important elements of the *AtPINOID* kinase binding to PA [79]. *AtSnRK2.4* contains multiple basic amino acid residues located in different regions, all involved in PA-binding [80]. In the case of the potassium channel *OsAKT2*, PA was found to bind two adjacent lysine residues in the cytosolic ANK domain [81] and basic residues in the S4 voltage-sensing domain. The cytosol-facing domains of *OsAKT2* contribute to the PA-mediated negative regulation of *OsAKT2* activity [82]. In addition, the EH1.1 domain of the TPC subunit *AtEH1/Pan1* (adaptor complex of clathrin-mediated endocytosis machinery) coordinates the PA molecules by three main regions (residues around K42, residues around K78 and residues located at the C-terminus around K97), whereas the EH1.2 domain interacts with the PA molecules at the region forming the phosphoinositide-binding site and at the region located close to the protein C-terminus. In contrast to EH1.1, EH1.2 binds a higher number of PA molecules [83].

The different affinities of PA species towards binding to target proteins may act as important determinants of PA signal specificity in response to dissimilar stimuli. The role of fatty acid tails in altering the affinity of PA towards a protein is likely determined by the specific structural features of the letter: e.g., the presence of a stretch of hydrophobic residues in the PA-binding region of protein phosphatase 2C *AtABI1* and protein kinase *AtCTR1* [32,84]; presence of hydrophobic residues at the C-terminus of *AtCP*, an actin-capping protein [85]; hydrophobic cavity in the structure of *AtSPHK1* [86]. PA is a cone-shaped molecule that induces negative spontaneous curvature [87], facilitating “deeper” insertion of proteins into lipid membranes, providing a background for the role of hydrophobic interactions [70]. On the other hand, some proteins, e.g., an MYB transcription factor (*AtWER*) [88] and glyceraldehyde-3-phosphate dehydrogenases (*AtGAPC1* and *AtGAPC2*) [89] do not show specific preferences for binding to particular PA species. For these proteins, binding is likely determined by the interaction of the phosphomonoester group of PA with basic residues.

Binding to PA is not necessarily associated with a tethering of proteins to PM. It has been shown that the binding of the cytosolic glyceraldehyde-3-phosphate dehydrogenase *AtGAPC2* to PA induces proteolytic cleavage of the enzyme [89]. It was found that PA interacts with the transcription factors *AtAHL4* (AT-hook motif-containing nuclear localized 4) [3] and *Arabidopsis* LATE ELONGATED HYPOCOTYL/CIRCADIAN CLOCK ASSOCIATED1 [92] and negatively regulates their DNA-binding ability. For *AtAHL4*, PA-mediated tethering to the nuclear membrane was excluded as a mechanism of PA action, suggesting a hypothetical possible association of PA with *AtAHL4* beyond membranes [3]. PA also binds to the gibberellin receptor *OsGID1*, promotes its nuclear localization, and mediates the gibberellin response in rice [36].

PA could regulate cell signaling by simultaneous binding to multiple components of the same regulatory pathway. One example is the binding of PA to the potassium channel *AtAKT2* [81] and *AtPP2CA* (protein phosphatase 2CA, negative modulator of *AtAKT2* activity) [93]. Similarly, PA binds to *AtMKK7/AtMKK9*, which phosphorylate PA-binding *AtMPK6* [94]; activates *AtMKK5*, and its downstream targets *AtMPK3/AtMPK6* [95]; binds *AtPINOID* kinase [79], and *Arabidopsis* D6 PROTEIN KINASE [96], all of which regulate PIN auxin transporter activity within the same pathway. PA has been shown to regulate not only the activity, phosphorylation status and/or localization of *AtSnRK2*, but also that of its cellular partners: *AtSnRK2* enzyme targets (e.g., *AtERD14*) and negative regulators (*AtABI1* and *AtSnRK2*-interacting calcium sensor) [97].

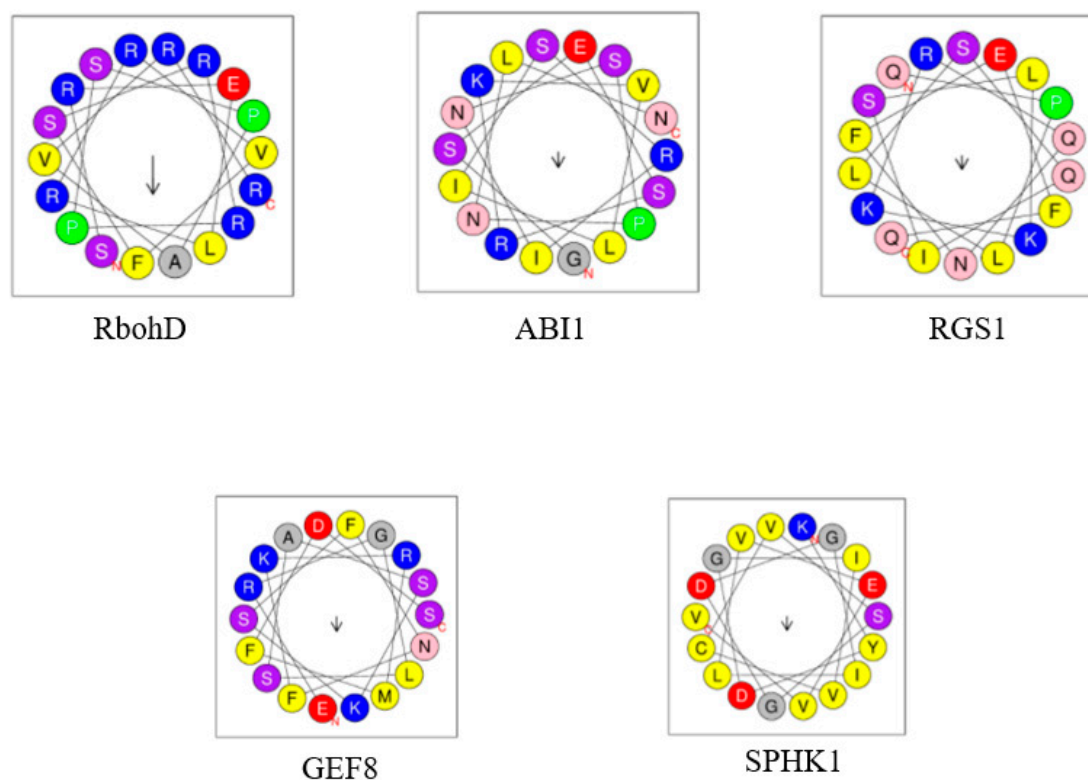


Figure 3. PA-binding folds of *Arabidopsis* proteins involved in plant hormone (abscisic acid) signaling. Shown are amphipathic α -helix projections of a minimal characteristic PA-binding domain obtained with “Heliquest” software [90]. The arrows indicate the hydrophobic moment. Basic and hydrophobic residues are shown in blue and yellow, respectively. PA-binding motifs were obtained from published data: AtRbohD [30], AtABI1 [32], AtRGS1 [91], AtGEF8 [75] and AtSPHK1 [86]. The sequences of PA-binding motifs are as follows: 140-SRELRRVFSRRPSPAVRRFD-159 (AtRbohD), 63-GSESRKVLISRINSPNLNMK-82 (AtABI), 250-QPLLSQISLKKRQNFQRM-269 (AtRGS1), 6-ERGLSASKSFNFKRMFDSSS-25 (AtGEF8), 175-KYDGIVCVSGDGILVEVVNG-194 (AtSPHK1).

5. Role of PA as a Lipid Second Messenger of Signal Transduction Pathways in Plants

5.1. Phosphatidic Acid Signaling in Hormonal Regulation

PA plays a key role in the regulation of metabolism in plants as well as in other living systems. PA is a mediator in the signal transduction pathways of hormones and other biologically active compounds that ensure plant growth, development and resistance to adverse environmental factors. Regulatory signals originating from hormones such as auxin, brassinosteroids, salicylic acid, abscisic acid, jasmonic acid and gibberellins have been found to implicate PA as a second messenger. PA, which is produced in response to the stimuli, has been found to be involved in genomic and non-genomic signaling events that regulate gene expression, ion transport, cytoskeletal dynamics and metabolic enzyme activities (Figure 4).

5.1.1. Role of Phosphatidic Acid in Auxin Signaling

Genetic studies suggest that the phospholipases AtPI-PLC2 [98] and AtNPC5 [99] play a role in auxin sensing pathways. PA binds to specific signaling proteins involved in the regulation of auxin transport and regulates their activity. For example, PA-binding 3'-phosphoinositide-dependent protein kinase1 (AtPDK1) phosphorylates and regulates PA-binding D6 protein kinase (AtD6PK) to stimulate the activity of PIN auxin efflux carriers [100]. PLD and PA were suggested to be involved in auxin-mediated reversion of the actin bundling induced by the hairpin elicitor [101].

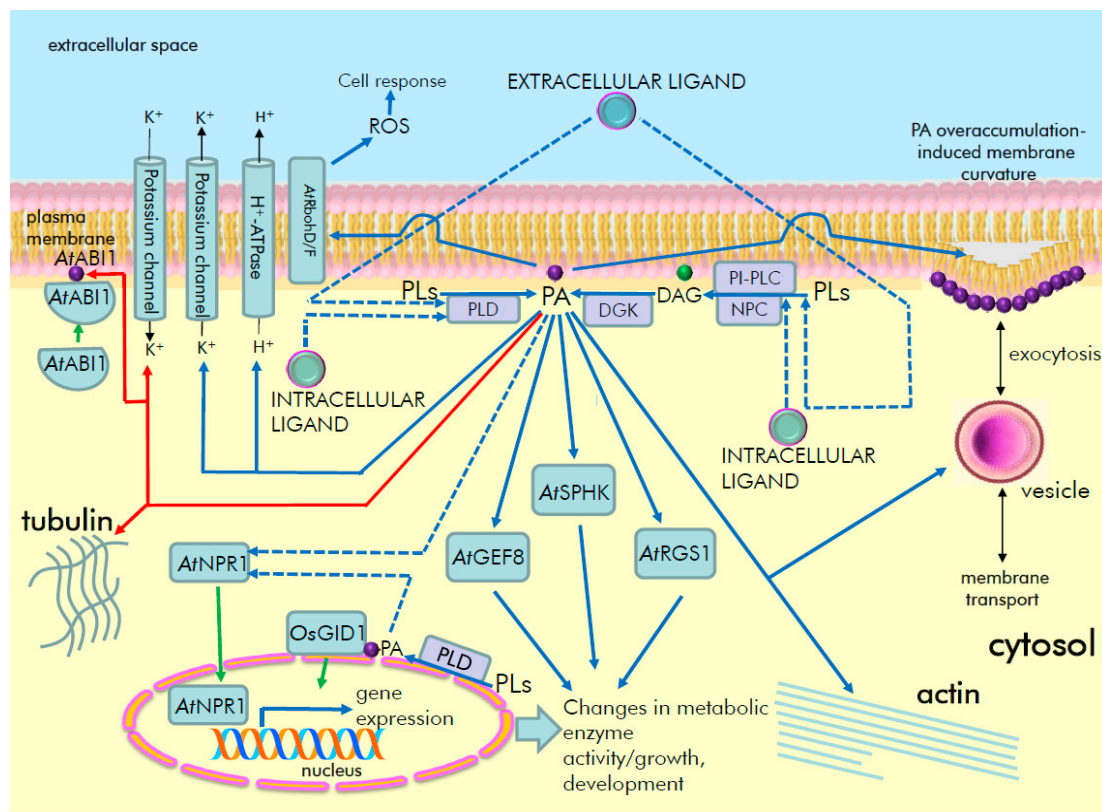


Figure 4. Schematic representation of the PA signaling in plants. Hypothetical receptors lead to activation of lipid signaling enzymes. Key examples of PA-regulated signaling events in hormonal regulation are shown (for details, see the main text); also shown here are PA-induced changes of membrane curvature and subsequent membrane transport events. Headgroups of DAG in a membrane are shown in green; headgroups of PA in crimson; and headgroups of other lipids in pink color. ABI1, ABSCISIC ACID-INSENSITIVE 1, *At*, *Arabidopsis thaliana*, DAG, diacylglycerol, DGK, diacylglycerol kinase, GEF8, RopGEF8, guanine nucleotide exchange factor 8, GID, GIBBERELLIN INSENSITIVE DWARF1, NPC, non-specific phospholipase C, NPR1, NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1, *Os*, *Oryza sativa*, PA, phosphatidic acid, PI-PLC, phosphatidylinositol-specific phospholipase C; PLD, phospholipase D, PLs, phospholipids (e.g., phosphatidylcholine, phosphatidylethanolamine), RbohD/F, respiratory burst oxidase protein D/F, RGS1, Regulator of G protein Signaling 1, ROS, reactive oxygen species, SPHK, sphingosine kinase.

5.1.2. Role of Phosphatidic Acid in Salicylic Acid Signaling

Salicylic acid (SA) activates PA production via DGK [102] and PLD [103–106] in *Arabidopsis thaliana* and *Capsicum chinense*. PLD and PA have been shown to be involved in transcriptome regulation by SA [105,107,108], SA-induced ROS accumulation via the NADPH oxidase AtRbohD [103], SA-induced metabolic changes [109] and nuclear translocation of AtNPR1, an SA signaling regulator [60].

5.1.3. Role of Phosphatidic Acid in Brassinosteroid Signaling

Brassinosteroids (BR) trigger the accumulation of DAG and PA derived from phosphatidylcholine in various plant tissues due to the activation of NPC and DGK [110–112]. AtNPC3 and AtNPC4 isozymes are involved in plant sensitivity to BR in terms of root growth and gene expression [110]. Several DGK isoforms contribute to the production of PA in response to BR. Epibrassinolide (EBL) treatment resulted in a two-fold increase in PA in WT *Arabidopsis thaliana* roots [113]. However, a similar response was reduced in *dgk1dgk2*, *dgk1* and *dgk5dgk6* mutant lines. Of all the *dgk* mutant lines tested, *dgk3* plants were characterized by the lowest PA induction in response to EBL treatment compared

with WT plants. Pretreatment of plants with BR further increased the levels of DAG and PA during subsequent salt stress. In this context, it has been suggested that DAG and PA are involved in the BR-induced adaptation of plants to high salinity [112]. Moreover, a reduced EBL-stimulated PA production in *dgk* mutants might be linked to lower germination rates, lower total and alternative respiration under optimal and high salinity conditions [113].

5.1.4. Role of Phosphatidic Acid in Abscisic Acid Signaling

Abscisic acid (ABA) is one of the most important plant hormones with respect to the regulation of plant metabolism (e.g., acting in seed germination, growth responses) and stress resistance (e.g., by controlling stomatal closure). ABA-induced rapid PA generation has been localized to the PM of *Arabidopsis thaliana* guard cells and cells of the root maturation zone [46]. PA plays a role as a second messenger in ABA-regulated events such as stomata closure and water stress tolerance. Specific PA-binding proteins are known to be involved in ABA signaling in plants. ABA-activated PLD leads to PA production, which inhibits AtRGS1—a regulator of G-protein signaling [91] and activates the guanine nucleotide exchange factor AtGEF8 [75]. Moreover, recruitment of ABI1 to the PM by the leucine-rich receptor-like kinase AtRDK1 in response to ABA requires the binding of PA to the former [114]. PLD and PA are also involved in ABA-induced microtubule depolymerization and calcium spikes in guard cells [115] and HVA1 expression [116].

The functional role of PA in ABA-signaling pathways could be illustrated by *in silico* analyses, which showed similarities in the gene expression patterns of ABA-treated wild-type plants and untreated *rgs1* lines. It is known that AtRGS1 is inhibited by PA as part of the ABA-signaling pathway [84]. Therefore, this signaling event is mimicked in *rgs1* plants, at least in part, by the absence of ABA. Comparing the two sets of gene expression data, we found that a number of genes encoding signaling, metabolic or stress-related proteins are regulated in a similar manner (Figure 5). These data suggest that inhibition of AtRGS1 by PA is indeed an important event in the ABA response in *Arabidopsis*.

5.1.5. Role of Phosphatidic Acid in Jasmonic Acid Signaling

Jasmonic acid is a hormone that rapidly activates the production of PA via PI-PLC/DGK and PLD in potato tubers [119], leaves of *Brassica napus* [120] and cells of *C. chinense* [102,121]. Jasmonate-induced accumulation of the secondary metabolites [102] and potato tuber cellular growth [119] were found to be downstream of PI-PLC\PLD activity and PA accumulation.

5.1.6. Role of Phosphatidic Acid in Gibberellin Signaling

Gibberellins were also suggested to interact with PA signaling. In rice, among several PLD isoforms tested, only OsPLD α 6 was found to be an important positive regulator of gibberellin sensitivity in terms of modulation of growth responses. Results from lipidomic analysis indicate that phosphatidylethanolamine and phosphatidylserine are the main sources of PA in response to gibberellin. In rice protoplasts, gibberellin induces OsPLD α 6 translocation from cytosol to nuclei. PA formed by OsPLD α 6 activity positively mediates gibberellin signaling via PA binding to gibberellin receptor OsGID1, promotion of its nuclear localization and degradation of the DELLA protein SLENDER RICE1, a negative regulator of the hormone signaling [36]. In contrast, the level of suppressor protein SLENDER RICE1 was decreased in *npc6* rice mutants treated with gibberellin, but drastically increased in OsNPC6 over-expressing plants [122]. Therefore, OsPLD α 6 and PA are positive players of early events of gibberellin signaling in plants.

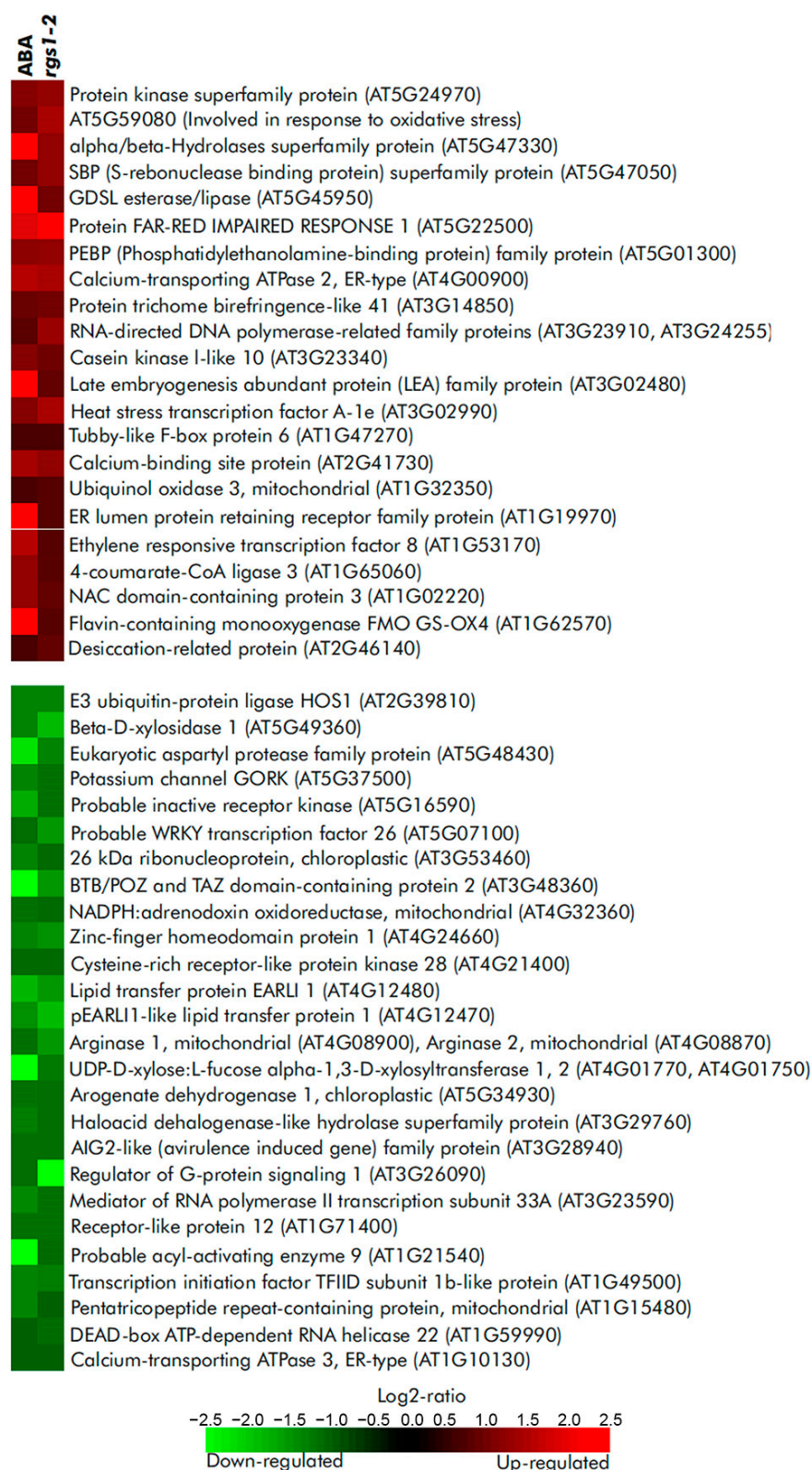


Figure 5. A set of similarly regulated genes in ABA-treated Col-0 *Arabidopsis* plants and in mock-treated *rgs1-2* knockout mutants obtained using “Geneinvestigator” database [55]. Experiments ID used to mine the data are as follows: ABA, AT-00433; *rgs1-2*, AT-00530. Changes in gene expression (\log_2 -ratio) are color-coded according to the provided scale. Statistical treatment of the data was implemented by “Geneinvestigator” database. Original data resources [117,118].

Hormones can also modulate PA signaling by controlling gene expression of PA-producing enzymes (Figure 6). Although the enzymes of PA turnover are well known, controlling the expression level of their encoding genes could still have a significant impact on the resulting signaling function. Thus, downregulation of *AtNPC6* expression following treatment with ABA could be a mechanism that attenuates the signaling response (Figure 6). Conversely, upregulation of *AtPLD δ* , *AtPLD ζ 2* and *AtDGK1* by the same stimuli could mean that the abundance of these isoenzymes is important to fulfill the latter steps in the ABA signaling pathway. On the other hand, cytokinins weakly affect PLD gene expression (Figure 6), but the role of PLD and PA as mediators of cytokinin-regulated gene expression was found [123,124].

5.2. Phosphatidic Acid as a Signal Transducer for Other Biologically Active Substances

Some hormone-like substances also trigger PA signaling. It has been shown that treatment with polyamines activates *AtPLD α 1* [125], *AtPLD δ* and DGK [52] in *Arabidopsis* seedlings and also PLD and DGK in *C. arabica* cells [126], leading to a rapid PA production. This response was dependent on the type of polyamines tested and their charge. For example, putrescine triggered higher *AtPLD α 1* activation in vitro compared to spermine and spermidine [125], while in *Arabidopsis* seedlings, spermine, thermospermine and spermidine induced higher PA accumulation compared to putrescine and diaminopropane [52]. Cellular uptake of spermine by the PM localized amino acid transporter Resistant to Methyl Viologen 1 (*AtRMV1*) is required for triggering PA production [52]. As a part of cell response to polyamines, *AtPLD α 1*-produced PA was shown to trigger H₂O₂ accumulation in guard cells [125], while *AtPLD δ* -produced PA triggers potassium ion efflux in the root elongation zone of *Arabidopsis* [53].

SCOOP12—an active endogenous plant peptide (phyto cytokine)—has been shown to rapidly stimulate the production of PA via the PI-PLC/DGK pathway in *Arabidopsis* cells [127]. During the self-incompatibility response of *Brassica napus* triggered by the interaction of SP11 peptide with SRK (S-locus receptor kinase), PLD α 1 was found to be a target of ARC1-mediated ubiquitination. Subsequent degradation of PLD α 1 thus impedes the production of PA, which itself is required for pollination. It has been proposed that activation of the PLD α 1 isoform by an unknown ligand during compatible pollination would lead to PM remodeling supported by the production of PA to promote exocytosis required for pollen germination [61]. Other data indirectly suggest a possible role of PA pathway enzymes in peptide signaling. *AtPI-PLC2* has been found to interact with *AtPXL1*—a receptor for the peptide *AtCLE41* [128]. Moreover, some peptide receptors might be co-expressed with PA-producing enzymes, as suggested by in silico analysis (see Table 2). This could indicate a possible functional link between certain peptide receptors and a signal lipid production.

PA signaling may also interact with signaling pathways of other bioactive substances. Both *AtPLD α 1* and *AtPLD δ* are involved in hydrogen sulfide signaling leading to stomatal closure in *Arabidopsis*. Hydrogen sulfide-induced H₂O₂ accumulation in guard cells was specifically dependent on *AtPLD α 1* but not on *AtPLD δ* . In contrast, hydrogen sulfide treatment led to PA accumulation in an *AtPLD δ* -dependent manner. Interestingly, hydrogen sulfide in *rbohD* lines did not induce PA accumulation in guard cells [129]. *RbohD* is an enzyme known to be regulated by PA [30], but in hydrogen sulfide signaling it, apparently, acts upstream of PLD-generated PA.

Extracellular *PbrS*-RNase, which is a proteinaceous female determinant of pear self-incompatibility, induces rapid PA accumulation that depends on *PbrPLD δ 1* expression in pollen tubes. PA thus prevents depolymerization of the actin cytoskeleton induced by *PbrS*-RNase and delays self-incompatibility signaling [130].

Biologically active substances can also modulate PA signaling by controlling gene expression of PA-producing enzymes (Figure 6). Consistent with this, the complex regulation of multiple PI-PLC and NPC genes by melatonin in soybean [131] supports the notion that bioregulators define an appropriate expression pattern of lipid signal transduction enzymes.

Table 2. Co-expression analysis of *Arabidopsis thaliana* genes coding selected peptide receptors and enzymes of PA signaling performed in silico using “Genevestigator” database [55]. All statistical data were implemented and provided by “Genevestigator” database; 1031 samples, 25 anatomical parts, 691 perturbations (AT_mRNASeq_ARABI), 10615 samples, 3243 perturbations, 7 developmental stages (AT_AFFY_ATH1) were analyzed. Pearson’s correlation coefficient (score) was used for statistical analysis of gene co-expression. Database dataset denotes aggregated expression data according to a specific category (samples, anatomy, development, perturbation).

Bioactive Peptide	Receptor	Co-Expressed PA-Metabolizing Enzyme	Platform	Database Dataset	Statistical Notes (Score)
PSK	PSKR1	PLDζ1	AT_mRNASeq_ARABI	anatomy	0.9
PSK	PSKR1	PLDγ1	AT_AFFY_ATH1	perturbation	0.69
PSK	PSKR1	PLDγ1	AT_AFFY_ATH1	sample	0.69
CEP1	CEPR1	PI-PLC3	AT_mRNASeq_ARABI	anatomy	0.89
CLE25	BAM2	NPC6	AT_AFFY_ATH1	sample	0.68
PIP1/2	RLK7	DGK5	AT_mRNASeq_ARABI	perturbation	0.85
PIP1/2	RLK7	PLDγ1	AT_mRNASeq_ARABI	sample	0.79
PEP	PEPR1	DGK1	AT_AFFY_ATH1	sample	0.56
PEP	PEPR2	DGK5	AT_mRNASeq_ARABI	sample	0.64
EPF2	TMM	NPC2	AT_AFFY_ATH1	development	0.9
LURE1	MIDS1	PI-PLC6	AT_mRNASeq_ARABI	perturbation	0.61
LURE1	MIK2	PLDγ1	AT_mRNASeq_ARABI	perturbation	0.82
LURE1	PRK8	PLDα2	AT_AFFY_ATH1	sample	0.82
LURE1	PRK3	DGK4	AT_mRNASeq_ARABI	perturbation	0.74
LURE1	PRK5	DGK4	AT_mRNASeq_ARABI	perturbation	0.74
GRIM REAPER	PRK5	DGK4	AT_mRNASeq_ARABI	perturbation	0.74
ESF1	SSP	DGK4	AT_AFFY_ATH1	sample	0.75
RALF1	BAK1	PLDγ1	AT_AFFY_ATH1	perturbation	0.73
RALF1	BAK1	PLDγ1	AT_mRNASeq_ARABI	sample	0.8
RALF1	BAK1	PLDγ1	AT_mRNASeq_ARABI	anatomy	0.85
Ralf4/19	BUPS1	DGK6	AT_AFFY_AG	sample	0.9
Ralf4/19	BUPS1	PI-PLC6	AT_mRNASeq_ARABI	sample	0.92
Ralf4/19	BUPS2	PI-PLC6	AT_mRNASeq_ARABI	sample	0.95
Ralf1/17/23/32/33	FERONIA	NPC1	AT_mRNASeq_ARABI	sample	0.7
Ralf1/17/23/32/33	FERONIA	PLDα1	AT_mRNASeq_ARABI	anatomy	0.88
Ralf1/17/23/32/33	FERONIA	PLDα1	AT_mRNASeq_ARABI	sample	0.79
Ralf1/17/23/32/33	FERONIA	PLDα1	AT_AFFY_ATH1	sample	0.5
Ralf4/19/34	ANXUR2	PLDα2	AT_AFFY_AGRONOMICS	sample	0.83
CLE45	SKM1	PLDε	AT_mRNASeq_ARABI	anatomy	0.61
CLE45	SKM1	PI-PLC5	AT_mRNASeq_ARABI	anatomy	0.57
CLE42	PXL2	PLDδ	AT_mRNASeq_ARABI	perturbation	0.71
TPD1	EMS1	PLDε	AT_AFFY_AGRONOMICS	perturbation	0.82

Abbreviations: BAK1, BRI1-associated kinase 1; BAM, BARELY ANY MERISTEM; BUPS, BUDDHA’S PAPER SEAL; CEP, C-TERMINALLY ENCODED PEPTIDE; CEPR, C-TERMINALLY ENCODED PEPTIDE RECEPTOR; CLE, CLAVATA3/EMBRYO SURROUNDING REGION; DGK, diacylglycerol kinase; EMS1, EXCESS MICROSPOROCTES1; EPF2, EPIDERMAL PATTERNING FACTOR 2; ESF1, EMBRYO SURROUNDING FACTOR1; MDIS, MALE DISCOVERER; MIK, MDIS1-INTERACTING RECEPTOR-LIKE KINASE; NPC, non-specific phospholipase C; PEP, PLANT ELICITOR PEPTIDE; PEPR, PLANT ELICITOR PEPTIDE RECEPTOR; PIP, PAMP-INDUCED SECRETED PEPTIDES; PI-PLC, phosphatidylinositol-specific phospholipase C; PLD, phospholipase D; PRK, POLLEN-SPECIFIC RECEPTOR-LIKE KINASE; PSK, PHYTOSULFOKINE; PSKR, PHYTOSULFOKINE RECEPTOR; PXL, PHLOEM INTERCALATED WITH XYLEM-LIKE; RALF, RAPID ALKALINIZATION FACTOR; RLK, RECEPTOR-LIKE KINASE; SKM, STERILITYREGULATING KINASE MEMBER; SSP, SHORT SUSPENSOR; TMM, TOO MANY MOUTHS; TPD1, TAPETUM DETERMINANT1.

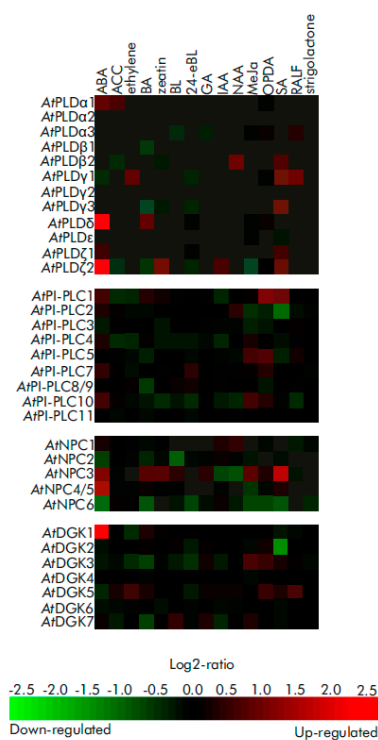


Figure 6. Meta-analysis of the expression pattern of PA-producing signaling enzymes (PLD, PI-PLC, NPC, DGK) in plants in response to phytohormones. Transcriptomic data were collected from the “Genevestigator” database [55] according to the outputs of classical array experiments (“AT_AFFY_ATH1” platform). Shown here is a heat-map of the relative values of gene expression differences between hormone-treated and mock-treated samples. All statistical data were implemented and provided by the “Genevestigator” database. Color saturation corresponds to the specific level of upregulation (red) and downregulation (green) of gene expression. Genes and corresponding AGI used: *AtPLDα1* (At3g15730), *AtPLDα2* (At1g52570), *AtPLDα3* (At5g25370), *AtPLDε* (At1g55180), *AtPLDβ1* (At2g42010), *AtPLDβ2* (At4g00240), *AtPLDγ1* (At4g11850), *AtPLDγ2* (At4g11830), *AtPLDγ3* (At4g11840), *AtPLDδ* (At4g35790), *AtPLDζ1* (At3g16785), *AtPLDζ2* (At3g05630); *AtPI-PLC1* (At5g58670), *AtPI-PLC2* (At3g08510), *AtPI-PLC3* (At4g38530), *AtPI-PLC4* (At5g58700), *AtPI-PLC5* (At5g58690), *AtPI-PLC7* (At3g55940), *AtPI-PLC8* (At3g47290), *AtPI-PLC9* (At3g47220), *AtPI-PLC10* (At4g34920), *AtPI-PLC11* (At3G19310); *AtNPC1* (At1g07230), *AtNPC2* (At2g26870), *AtNPC3* (At3g03520), *AtNPC4* (At3g03530), *AtNPC5* (At3g03540), *AtNPC6* (At3g48610); *AtDGK1* (At5g07920), *AtDGK2* (At5g63770), *AtDGK3* (At2g18730), *AtDGK4* (At5g57690), *AtDGK5* (At2g20900), *AtDGK6* (At4g28130), *AtDGK7* (At4g30340). Gene of *AtPI-PLC6* (At2g40116) was not represented in the corresponding platform. A total of 15 experiments were used for the analysis. Abbreviations, short description of experimental conditions (time of treatment, concentrations, tissue types, genotypes) and experiment ID codes for each set of experimental data (from figure left to right) are as follows: ABA, abscisic acid, 10 μM, 3 h, seedlings, Col-0 (AT-00110); ACC, 1-aminocyclopropane-1-carboxylic acid, 10 μM, 3 h, seedlings, Col-0 (AT-00110); ethylene, 5 ppm, 3 h, petiole samples, Col-0 (AT-00013); BA, 6-benzylaminopurine, 10 μM, 1 h, shoot samples, Col-0 (AT-00351); zeatin, 1 μM, 3 h, seedlings, Col-0 (AT-00110); BL, brassinolide, 10 nM, 3 h, seedlings, Col-0 (AT-00110); 24-eBL, 24-epibrassinolide, 0.1 μM, 3 h, dark, seedling sample, Col (AT-00650); GA, gibberellic acid 3, 1 μM, 3 h, seedlings, Col-0 (AT-00110); IAA, indole-acetic acid, 1 μM, 3 h, seedlings, Col-0 (AT-00110); NAA, naphthaleneacetic acid (a synthetic auxin), 10 μM, 2 h, leaf disc samples, Col-0 (AT-00392); MeJa, methyl jasmonate, 10 μM, 3 h, seedlings, Col-0 (AT-00110); OPDA, 12-oxo-phytodienoic acid, 75 μM, 4 h, seedlings, Col-0 (AT-00293); SA, salicylic acid, 2 mM, 1 day, seedlings, Col-0 (AT-00320); RALF, Rapid Alkalinization Factor 1 (peptide, *AtRALF*), 1 μM, 30 min, seedling sample, Col (AT-00679); strigolactone, GR24, 1 μM, 90 min, *max3-9* (a strigolactone deficient mutant) whole plant samples (AT-00404). Original data resources: GSE14961/E-GEOD-14961 datasets for salicylic acid-regulated gene expression (AT-00320), [132–139] for other hormones/biologically active substances.

6. The Most Common Methods for the Studying of Phosphatidic Acid Signaling in Plants

The detection of transient and minute changes in PA production in plants requires a specific methodological strategy. Current approaches for the measuring of PA content include radioisotope labeling, chromatographic techniques and mass-spectrometric methods [140,141]. PA changes are often rapid, requiring appropriate timing and rigorous handling, extraction and determination of PA, which are time-consuming and expensive.

Recent insights into the nature of protein-phospholipid interactions have enabled the development of genetically-encoded fluorescent molecular probes that can specifically interact with various phospholipids and allow their visualization in living cells. To this end, PA-specific probes containing a PA-binding domain are fused with fluorescent proteins and expressed in plant cells. For example, Potocky et al. [41] constructed a PA biosensor consisting of a Spo20p-PA-binding domain fused with a fluorescent YFP protein that reflects the dynamics of PA in transiently transformed tobacco pollen tubes, as shown by confocal laser scanning microscopy [41]. Subsequently, Li et al. developed a Förster resonance energy transfer technology (FRET)-based PA-specific biosensor PAleon that monitored and visualized the concentration and dynamics of PA in plant cells under abiotic stress [46]. These PA biosensors have helped to reveal the spatiotemporal dynamics of PA in plants. Similarly, DAG, PS, PIP₂ and PIP lipids biosensors have also been successfully used in plant cells [142]. These biosensors can be used for real-time monitoring of lipid dynamics and providing new insights into lipid metabolism and signaling in plants.

The PA content in plant tissue extracts can also be measured using a PA enzyme-linked immunosorbent assay (ELISA) with an anti-PA antibody, where the PA content is calculated using a standard curve [143]. In addition, a fluorometrically-coupled enzyme assay for measuring PA is also known. It involves PA deacylation by added lipase and further oxidation of PA-derived glycerol-3-phosphate to generate hydrogen peroxide. The latter is subsequently used for the peroxidase-catalyzed conversion of Amplex Red reagent to a fluorescent resorufin. The fluorescence intensity of resorufin is then measured using a fluorescence microplate reader [144].

7. Conclusions

Despite the well-documented role of PA as a biologically active molecule, we still lack an understanding of the underlying mechanisms. In particular, it is still not clear how the enzymes of PA production in the PM are rapidly activated (usually within minutes) in response to hormones. Not all hormone receptors are located in the PM, so a spatial relationship can be inferred; subsequent studies will be required to produce a more complete outlook.

Deciphering the role of PA in the regulation of multiple cellular activities remains to be a challenge for future research. For example, under nutrient limitation, cells induce autophagy that delivers cellular cargoes for degradation as a part of the nutrient recycling mechanism. The role of PA in plant autophagy could be inferred from data on plant [145] and animal [146] systems. In *Arabidopsis*, *AtPLDε* has been found to promote autophagy in plant response to nitrogen deficiency. Physiological analysis indicates that *AtPLDε* acts to delay senescence in response to nitrogen deficiency. The transcript level of *AtPLDε* was increased in these stress conditions, while analysis of *AtPLDε*-GFP cellular distribution indicated that nitrate starvation increased protein levels of *AtPLDε* [145] and promoted its association with intracellular membranes. Co-immunoprecipitation and bimolecular fluorescence complementation analysis indicate that *AtPLDε* interacts with *AtATG8*, an autophagy-related protein that is essential for the biogenesis, cargo recruitment and maturation of autophagosomes. Phosphatidylethanolamine (PE) lipidation of *AtATG8* is necessary to its functions; an incubation of affinity-purified protein *AtPLDε* with membrane proteins in plants expressing GFP-*AtATG8* indicated that *AtPLDε* delipidates *AtATG8*-PE complex. Results of immunoblotting suggested that during nitrogen starvation, *AtPLDε* increases the abundance of the *AtATG8*-PE complex. Genetic analysis of GFP-*AtATG8*-labeled *AtPLDε*-mutated plants demonstrated that *AtPLDε* promotes autophagosome formation in response

to nitrogen deprivation. Monitoring of the levels of *AtATG1a* and *AtNBR1* (NEIGHBOR OF BRCA1) proteins that are known to be degraded by autophagy under stress suggested that *AtPLD ϵ* increases their autophagic degradation under nitrogen deficiency [145]. Therefore, PLD and PA could play a multifaced role in plant autophagy.

The mechanisms and specificity of binding of PA to proteins are also not fully understood. Despite a proposed mechanism primarily involving a phosphomonoester group of PA (hydrogen bond switch model), a role for the variable fatty acid tails of PA in protein binding is apparent. More experimental evidence needs to be collected before the full PA-binding properties of proteins can be considered.

Another unsolved problem is to link the signaling function of PA with a metabolic one. PA is a critical intermediate in lipid turnover and plays a role in recycling orthophosphate into sink tissues, thus minimizing phosphate starvation [147,148]. Furthermore, a basal PA deficiency in *pld α 1* apparently leads to altered abundances of proteins involved in various housekeeping activities [149,150].

Moreover, not only the increase in PA content might have a regulatory effect. The rapid decrease of basal PA by abscisic acid and salicylic acid in *Arabidopsis* cells [107] raises the question of whether it also has a signaling function.

An intriguing fact is the PA's ability to bind transcription factors and negatively regulate their DNA binding [3,92]. This suggests that the abundance of PA in the nuclear membrane may be a factor controlling the transcriptome through a molecular interface yet to be identified.

Author Contributions: Y.K., S.K., Y.B., I.P., E.R., J.M. and V.K. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by grants from NAS Ukraine (N 13-03-20/21; 2.1.10.32-20) and the Ministry of Education, Youth and Sports of the Czech Republic (European Regional Development Fund—Project 'Centre for Experimental Plant Biology' No. CZ.02.1.01/0.0/0.0/16_019/0000738).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kwiatek, J.M.; Carman, G.M. Yeast phosphatidic acid phosphatase Pah1 hops and scoots along the membrane phospholipid bilayer. *J. Lipid Res.* **2020**, *61*, 1232–1243. [CrossRef] [PubMed]
2. Zhukovsky, M.A.; Filograna, A.; Luini, A.; Corda, D.; Valente, C. Phosphatidic acid in membrane rearrangements. *FEBS Lett.* **2019**, *593*, 2428–2451. [CrossRef] [PubMed]
3. Cai, G.; Kim, S.-C.; Li, J.; Zhou, Y.; Wang, X. Transcriptional regulation of lipid catabolism during seedling establishment. *Mol. Plant* **2020**, *13*, 984–1000. [CrossRef]
4. Zhang, G.; Yang, J.; Chen, X.; Zhao, D.; Zhou, X.; Zhang, Y.; Wang, X.; Zhao, J. Phospholipase D- and phosphatidic acid-mediated phospholipid metabolism and signaling modulate symbiotic interaction and nodulation in soybean (*Glycine max*). *Plant J.* **2021**, *106*, 142–158. [CrossRef]
5. Lavell, A.A.; Benning, C. Cellular organization and regulation of plant glycerolipid metabolism. *Plant Cell Physiol.* **2019**, *60*, 1176–1183. [CrossRef]
6. Pokotylo, I.; Kravets, V.; Martinec, J.; Ruelland, E. The phosphatidic acid paradox: Too many actions for one molecule class? Lessons from plants. *Progress Lipid Res.* **2018**, *71*, 43–53. [CrossRef]
7. Li, J.; Wang, X. Phospholipase D and phosphatidic acid in plant immunity. *Plant Sci.* **2019**, *279*, 45–50. [CrossRef]
8. Meijer, H.J.G.; van Himbergen, J.A.J.; Musgrave, A.; Munnik, T. Acclimation to salt modifies the activation of several osmotic stress-activated lipid signalling pathways in *Chlamydomonas*. *Phytochemistry* **2017**, *135*, 64–72. [CrossRef]
9. Yao, Y.; Li, J.; Lin, Y.; Zhou, J.; Zhang, P.; Xu, Y. Structural insights into phospholipase D function. *Prog. Lipid Res.* **2021**, *81*, 101070. [CrossRef]
10. Kocourková, D.; Krčková, Z.; Pejchar, P.; Kroumanová, K.; Podmanická, T.; Daněk, M.; Martinec, J. Phospholipase D α 1 mediates the high-Mg²⁺ stress response partially through regulation of K⁺ homeostasis. *Plant Cell Environ.* **2020**, *43*, 2460–2475. [CrossRef]
11. Kolesnikov, Y.S.; Nokhrina, K.P.; Kretynin, S.V.; Volotovskii, I.D.; Martinec, J.; Romanov, G.A.; Kravets, V.S. Molecular structure of phospholipase D and regulatory mechanisms of its activity in plant and animal cells. *Biochemistry* **2012**, *77*, 1–14. [CrossRef] [PubMed]
12. Kocourková, D.; Kroumanová, K.; Podmanická, T.; Daněk, M.; Martinec, J. Phospholipase D α 1 acts as a negative regulator of high Mg²⁺-induced leaf senescence in *Arabidopsis*. *Front. Plant Sci.* **2021**, *12*, 770794. [CrossRef] [PubMed]

13. Zhang, Y.; Gao, Z.; Hu, M.; Pan, Y.; Xu, X.; Zhang, Z. Delay of ripening and senescence in mango fruit by 6-benzylaminopurine is associated with inhibition of ethylene biosynthesis and membrane lipid catabolism. *Postharvest Biol. Technol.* **2022**, *185*, 111797. [[CrossRef](#)]
14. Arisz, S.A.; Testerink, C.; Munnik, T. Plant PA signaling via diacylglycerol kinase. *Biochim. Biophys. Acta* **2009**, *1791*, 869–875. [[CrossRef](#)]
15. Yuan, S.; Kim, S.-C.; Deng, X.; Hong, Y.; Wang, X. Diacylglycerol kinase and associated lipid mediators modulate rice root architecture. *New Phytol.* **2019**, *223*, 261–276. [[CrossRef](#)]
16. Kue Foka, I.C.; Ketehouli, T.; Zhou, Y.; Li, X.-W.; Wang, F.-W.; Li, H. The emerging roles of diacylglycerol kinase (DGK) in plant stress tolerance, growth, and development. *Agronomy* **2020**, *10*, 1375. [[CrossRef](#)]
17. Nakamura, Y.; Ngo, A.H. Non-specific phospholipase C (NPC): An emerging class of phospholipase C in plant growth and development. *J. Plant Res.* **2020**, *133*, 489–497. [[CrossRef](#)]
18. Sagar, S.; Singh, A. Emerging role of phospholipase C mediated lipid signaling in abiotic stress tolerance and development in plants. *Plant Cell Rep.* **2021**, *40*, 2123–2133. [[CrossRef](#)]
19. Craddock, C.P.; Adams, N.; Kroon, J.T.M.; Bryant, F.M.; Hussey, P.J.; Kurup, S.; Eastmond, P.J. Cyclin-dependent kinase activity enhances phosphatidylcholine biosynthesis in Arabidopsis by repressing phosphatidic acid phosphohydrolase activity. *Plant J.* **2017**, *89*, 3–14. [[CrossRef](#)]
20. Su, W.; Raza, A.; Zeng, L.; Gao, A.; Lv, Y.; Ding, X.; Cheng, Y.; Zou, X. Genome-wide analysis and expression patterns of lipid phospholipid phospholipase gene family in *Brassica napus* L. *BMC Genom.* **2021**, *22*, 548. [[CrossRef](#)]
21. Zhou, Z.; Yang, Y.; Shan, W.; Zhang, H.; Wei, W.; Kuang, J.; Chen, J.; Lu, W. Ethylene attenuates chilling injury of banana fruit via the MabHLH060/183 module in controlling phosphatidic acid formation genes. *Postharvest Biol. Technol.* **2022**, *183*, 111724. [[CrossRef](#)]
22. Takáč, T.; Novák, D.; Šamaj, J. Recent advances in the cellular and developmental biology of phospholipases in plants. *Front. Plant Sci.* **2019**, *10*, 362. [[CrossRef](#)] [[PubMed](#)]
23. Tan, Y.; Wang, L. MpDGK2, a novel diacylglycerol kinase from *Malus prunifolia*, confers drought stress tolerance in transgenic Arabidopsis. *Plant Mol. Biol. Report.* **2020**, *38*, 452–460. [[CrossRef](#)]
24. Rodas-Junco, B.A.; Racagni-Di-Palma, G.E.; Canul-Chan, M.; Usorach, J.; Hernández-Sotomayor, S.M.T. Link between lipid second messengers and osmotic stress in plants. *Int. J. Mol. Sci.* **2021**, *22*, 2658. [[CrossRef](#)]
25. Zhang, Q.; van Wijk, R.; Shahbaz, M.; Roels, W.; Schooten, B.V.; Vermeer, J.E.M.; Zarza, X.; Guardia, A.; Scuffi, D.; García-Mata, C.; et al. Arabidopsis phospholipase C3 is involved in lateral root initiation and ABA responses in seed germination and stomatal closure. *Plant Cell Physiol.* **2018**, *59*, 469–486. [[CrossRef](#)]
26. Zhu, L.; Dou, L.; Shang, H.; Li, H.; Yu, J.; Xiao, G. GhPIPLC2D promotes cotton fiber elongation by enhancing ethylene biosynthesis. *iScience* **2021**, *24*, 102199. [[CrossRef](#)]
27. Kong, X.-X.; Mei, J.-W.; Zhang, J.; Liu, X.; Wu, J.-Y.; Wang, C.-L. Turnover of diacylglycerol kinase 4 by cytoplasmic acidification induces vacuole morphological change and nuclear DNA degradation in the early stage of pear self-incompatibility response. *J. Integr. Plant Biol.* **2021**, *63*, 2123–2135. [[CrossRef](#)]
28. Zhang, Y.; Liu, R.; Zhou, Y.; Wang, S.; Zhang, B.; Kong, J.; Zheng, S.; Yang, N. PLD α 1 and GPA1 are involved in the stomatal closure induced by oridonin in *Arabidopsis thaliana*. *Funct. Plant Biol.* **2021**, *48*, 1005–1016. [[CrossRef](#)]
29. Jia, Y.; Li, W. Characterisation of lipid changes in ethylene-promoted senescence and its retardation by suppression of phospholipase D δ in *Arabidopsis* leaves. *Front. Plant Sci.* **2015**, *6*, 1045. [[CrossRef](#)]
30. Zhang, Y.; Zhu, H.; Zhang, Q.; Li, M.; Yan, M.; Wang, R.; Wang, L.; Welti, R.; Zhang, W.; Wang, X. Phospholipase D α 1 and phosphatidic acid regulate NADPH oxidase activity and production of reactive oxygen species in ABA-mediated stomatal closure in *Arabidopsis*. *Plant Cell* **2009**, *21*, 2357–2377. [[CrossRef](#)]
31. Guo, L.; Devaiah, S.P.; Narasimhan, R.; Pan, X.; Zhang, Y.; Zhang, W.; Wang, X. Cytosolic glyceraldehyde-3-phosphate dehydrogenases interact with phospholipase D δ to transduce hydrogen peroxide signals in the *Arabidopsis* response to stress. *Plant Cell* **2012**, *24*, 2200–2212. [[CrossRef](#)] [[PubMed](#)]
32. Zhang, W.; Qin, C.; Zhao, J.; Wang, X. Phospholipase D α 1-derived phosphatidic acid interacts with ABI1 phosphatase 2C and regulates abscisic acid signaling. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 9508–9513. [[CrossRef](#)] [[PubMed](#)]
33. Guo, L.; Mishra, G.; Taylor, K.; Wang, X. Phosphatidic acid binds and stimulates *Arabidopsis* sphingosine kinases. *J. Biol. Chem.* **2011**, *286*, 13336–13345. [[CrossRef](#)] [[PubMed](#)]
34. Wei, F.; Fanella, B.; Guo, L.; Wang, X. Membrane glycerolipidome of soybean root hairs and its response to nitrogen and phosphate availability. *Sci. Rep.* **2016**, *6*, 36172. [[CrossRef](#)] [[PubMed](#)]
35. Vismans, G.; van der Meer, T.; Langevoort, O.; Schreuder, M.; Bouwmeester, H.; Peisker, H.; Dörman, P.; Ketelaar, T.; van der Krol, A. Low-phosphate induction of plastidal stromules is dependent on strigolactones but not on the canonical strigolactone signaling component MAX2. *Plant Physiol.* **2016**, *172*, 2235–2244. [[CrossRef](#)]
36. Cao, H.; Gong, R.; Yuan, S.; Su, Y.; Lv, W.; Zhou, Y.; Zhang, Q.; Deng, X.; Tong, P.; Liang, S.; et al. Phospholipase D α 6 and phosphatidic acid regulate gibberellin signaling in rice. *EMBO Rep.* **2021**, *22*, e51871. [[CrossRef](#)] [[PubMed](#)]
37. Sakane, F.; Hoshino, F.; Murakami, C. New era of diacylglycerol kinase, phosphatidic acid and phosphatidic acid-binding protein. *Int. J. Mol. Sci.* **2020**, *21*, 6794. [[CrossRef](#)]

38. McDermott, M.I.; Wang, Y.; Wakelam, M.J.O.; Bankaitis, V.A. Mammalian phospholipase D: Function, and therapeutics. *Prog. Lipid Res.* **2020**, *78*, 101018. [[CrossRef](#)]
39. Lutkewitte, A.J.; Finck, B.N. Regulation of signaling and metabolism by lipin-mediated phosphatidic acid phosphohydrolase activity. *Biomolecules* **2020**, *10*, 1386. [[CrossRef](#)]
40. Graber, Z.; Owusu Kwarteng, D.; Lange, S.M.; Koukanas, Y.; Khalifa, H.; Mutambuze, J.W.; Kooijman, E.E. The electrostatic basis of diacylglycerol pyrophosphate-protein interaction. *Cells* **2022**, *11*, 290. [[CrossRef](#)]
41. Potocký, M.; Pleskot, R.; Pejchar, P.; Vitale, N.; Kost, B.; Zarsky, V. Live-cell imaging of phosphatidic acid dynamics in pollen tubes visualized by Spo20p-derived biosensor. *New Phytol.* **2014**, *203*, 483–494. [[CrossRef](#)] [[PubMed](#)]
42. Pejchar, P.; Sekereš, J.; Novotný, O.; Žárský, V.; Potocký, M. Functional analysis of phospholipase D δ family in tobacco pollen tubes. *Plant J.* **2020**, *103*, 212–226. [[CrossRef](#)] [[PubMed](#)]
43. Kubátová, Z.; Pejchar, P.; Potocký, M.; Sekereš, J.; Žárský, V.; Kulich, I. *Arabidopsis* trichome contains two plasma membrane domains with different lipid compositions which attract distinct EXO70 subunits. *Int. J. Mol. Sci.* **2019**, *20*, 3803. [[CrossRef](#)] [[PubMed](#)]
44. Platre, M.P.; Noack, L.C.; Doumane, M.; Bayle, V.; Simon, M.L.A.; Maneta-Peyret, L.; Fouillen, L.; Stanislas, T.; Armengot, L.; Pejchar, P.; et al. A combinatorial lipid code shapes the electrostatic landscape of plant endomembranes. *Dev. Cell* **2018**, *45*, 465–480.e11. [[CrossRef](#)] [[PubMed](#)]
45. Hertle, A.P.; García-Cerdán, J.G.; Armbruster, U.; Shih, R.; Lee, J.J.; Wong, W.; Niyogi, K.K. A Sec14 domain protein is required for photoautotrophic growth and chloroplast vesicle formation in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 9101–9111. [[CrossRef](#)]
46. Li, W.; Song, T.; Wallrad, L.; Kudla, J.; Wang, X.; Zhang, W. Tissue-specific accumulation of pH-sensing phosphatidic acid determines plant stress tolerance. *Nat. Plants* **2019**, *5*, 1012–1021. [[CrossRef](#)]
47. Wang, Y.-S.; Yao, H.-Y.; Xue, H.-W. Lipidomic profiling analysis reveals the dynamics of phospholipid molecules in *Arabidopsis thaliana* seedling growth. *J. Integr. Plant Biol.* **2016**, *58*, 890–902. [[CrossRef](#)]
48. Devaiah, S.P.; Roth, M.R.; Baughman, E.; Li, M.; Tamura, P.; Jeannotte, R.; Welti, R.; Wang, X. Quantitative profiling of polar glycerolipid species from organs of wild-type *Arabidopsis* and a phospholipase D α 1 knockout mutant. *Phytochemistry* **2006**, *67*, 1907–1924. [[CrossRef](#)]
49. Yunus, I.S.; Cazenave-Gassiot, A.; Liu, Y.-C.; Lin, Y.-C.; Wenk, M.R.; Nakamura, Y. Phosphatidic acid is a major phospholipid class in reproductive organs of *Arabidopsis thaliana*. *Plant Signal. Behav.* **2015**, *10*, e1049790. [[CrossRef](#)]
50. Nakamura, Y.; Teo, N.Z.; Shui, G.; Chua, C.H.; Cheong, W.F.; Parameswaran, S.; Koizumi, R.; Ohta, H.; Wenk, M.R.; Ito, T. Transcriptomic and lipidomic profiles of glycerolipids during *Arabidopsis* flower development. *New Phytol.* **2014**, *203*, 310–322. [[CrossRef](#)]
51. Arisz, S.A.; van Wijk, R.; Roels, W.; Zhu, J.-K.; Haring, M.A.; Munnik, T. Rapid phosphatidic acid accumulation in response to low temperature stress in *Arabidopsis* is generated through diacylglycerol kinase. *Front. Plant Sci.* **2013**, *4*, 1. [[CrossRef](#)] [[PubMed](#)]
52. Zarza, X.; Shabala, L.; Fujita, M.; Shabala, S.; Haring, M.A.; Tiburcio, A.F.; Munnik, T. Extracellular spermine triggers a rapid intracellular phosphatidic acid response in *Arabidopsis*, involving PLD δ activation and stimulating ion flux. *Front. Plant Sci.* **2019**, *10*, 601. [[CrossRef](#)] [[PubMed](#)]
53. Hu, X.; Wang, H.; Li, K.; Wu, Y.; Liu, Z.; Huang, C. Genome-wide proteomic profiling reveals the role of dominance protein expression in heterosis in immature maize ears. *Sci. Rep.* **2017**, *7*, 16130. [[CrossRef](#)] [[PubMed](#)]
54. Liu, G.-J.; Xiao, G.-H.; Liu, N.-J.; Liu, D.; Chen, P.-S.; Qin, Y.-M.; Zhu, Y.-X. Targeted lipidomics studies reveal that linolenic acid promotes cotton fiber elongation by activating phosphatidylinositol and phosphatidylinositol monophosphate biosynthesis. *Mol. Plant* **2015**, *8*, 911–921. [[CrossRef](#)] [[PubMed](#)]
55. Hruz, T.; Laule, O.; Szabo, G.; Wessendorp, F.; Bleuler, S.; Oertle, L.; Widmayer, P.; Gruissem, W.; Zimmermann, P. Genevestigator V3: A reference expression database for the meta-analysis of transcriptomes. *Adv. Bioinform.* **2008**, *2008*, 420747. [[CrossRef](#)]
56. Stenzel, I.; Ischebeck, T.; Vu-Becker, L.H.; Riechmann, M.; Krishnamoorthy, P.; Fratini, M.; Heilmann, I. Coordinated localization and antagonistic function of NtPLC3 and PI4P 5-Kinases in the subapical plasma membrane of tobacco pollen tubes. *Plants* **2020**, *9*, 452. [[CrossRef](#)]
57. Han, X.; Shi, Y.; Liu, G.; Guo, Y.; Yang, Y. Activation of ROP6 GTPase by phosphatidylglycerol in *Arabidopsis*. *Front. Plant Sci.* **2018**, *9*, 347. [[CrossRef](#)]
58. de Jong, F.; Munnik, T. Attracted to membranes: Lipid-binding domains in plants. *Plant Physiol.* **2021**, *185*, 707–723. [[CrossRef](#)]
59. Hempel, F.; Stenzel, I.; Heilmann, M.; Krishnamoorthy, P.; Menzel, W.; Golbik, R.; Helm, S.; Dobritzsch, D.; Baginsky, S.; Lee, J.; et al. MAPKs influence pollen tube growth by controlling the formation of phosphatidylinositol 4,5-bisphosphate in an apical plasma membrane domain. *Plant Cell* **2017**, *29*, 3030–3050. [[CrossRef](#)]
60. Janda, M.; Šašek, V.; Chmelařová, H.; Andrejch, J.; Nováková, M.; Hajšlová, J.; Burketová, L.; Valentová, O. Phospholipase D affects translocation of NPR1 to the nucleus in *Arabidopsis thaliana*. *Front. Plant Sci.* **2015**, *6*, 59. [[CrossRef](#)]
61. Scandola, S.; Samuel, M.A. A flower-specific phospholipase D is a stigmatic compatibility factor targeted by the self-incompatibility response in *Brassica napus*. *Curr. Biol.* **2019**, *29*, 506–512.e4. [[CrossRef](#)]
62. Pleskot, R.; Pejchar, P.; Bezvoda, R.; Lichtscheidl, I.; Wolters-Arts, M.; Marc, J.; Žárský, V.; Potocký, M. Turnover of phosphatidic acid through distinct signaling pathways affects multiple aspects of pollen tube growth in tobacco. *Front. Plant Sci.* **2012**, *3*, 54. [[CrossRef](#)] [[PubMed](#)]

63. Galvan-Ampudia, C.S.; Julkowska, M.M.; Darwish, E.; Gandullo, J.; Korver, R.A.; Brunoud, G.; Haring, M.A.; Munnik, T.; Vernoux, T.; Testerink, C. Halotropism Is a Response of Plant Roots to Avoid a Saline Environment. *Curr. Biol.* **2013**, *23*, 2044–2050. [[CrossRef](#)] [[PubMed](#)]
64. Vaz Dias, F.; Serrazina, S.; Vitorino, M.; Marchese, D.; Heilmann, I.; Godinho, M.; Rodrigues, M.; Malhó, R. A role for diacylglycerol kinase 4 in signalling crosstalk during *Arabidopsis* pollen tube growth. *New Phytol.* **2019**, *222*, 1434–1446. [[CrossRef](#)] [[PubMed](#)]
65. Wattelet-Boyer, V.; Le Guédard, M.; Dittrich-Domergue, F.; Maneta-Peyret, L.; Kriechbaumer, V.; Boutté, Y.; Bessoule, J.-J.; Moreau, P. Lysophosphatidic acid acyltransferases: A link with intracellular protein trafficking in *Arabidopsis* root cells? *J. Exp. Bot.* **2021**, *73*, 1327–1343. [[CrossRef](#)] [[PubMed](#)]
66. Novák, D.; Vadovič, P.; Ovečka, M.; Šamajová, O.; Komis, G.; Colcombet, J.; Šamaj, J. Gene expression pattern and protein localization of *Arabidopsis* phospholipase D alpha 1 revealed by advanced light-sheet and super-resolution microscopy. *Front. Plant Sci.* **2018**, *9*, 371. [[CrossRef](#)] [[PubMed](#)]
67. Kaneda, M.; van Oostende-Triplet, C.; Chebli, Y.; Testerink, C.; Bednarek, S.Y.; Geitmann, A. Plant AP180 N-Terminal Homolog proteins are involved in clathrin-dependent endocytosis during pollen tube growth in *Arabidopsis thaliana*. *Plant Cell Physiol.* **2019**, *60*, 1316–1330. [[CrossRef](#)] [[PubMed](#)]
68. McLoughlin, F.; Arisz, S.A.; Dekker, H.L.; Kramer, G.; De Koster, C.G.; Haring, M.A.; Munnik, T.; Testerink, C. Identification of novel candidate phosphatidic acid-binding proteins involved in the salt-stress response of *Arabidopsis thaliana* roots. *Biochem. J.* **2013**, *450*, 573. [[CrossRef](#)] [[PubMed](#)]
69. Sekereš, J.; Pejchar, P.; Šantrůček, J.; Vukašinić, N.; Žárský, V.; Potocký, M. Analysis of exocyst subunit EXO70 family reveals distinct membrane polar domains in tobacco pollen tubes. *Plant Physiol.* **2017**, *173*, 1659–1675. [[CrossRef](#)] [[PubMed](#)]
70. Putta, P.; Rankenberg, J.; Korver, R.A.; van Wijk, R.; Munnik, T.; Testerink, C.; Kooijman, E.E. Phosphatidic acid binding proteins display differential binding as a function of membrane curvature stress and chemical properties. *Biochim. Biophys. Acta (BBA)-Biomembr.* **2016**, *1858*, 2709–2716. [[CrossRef](#)]
71. Putta, P.; Creque, E.; Piontkivska, H.; Kooijman, E.E. Lipid-protein interactions for ECA1 an N-ANTH domain protein involved in stress signaling in plants. *Chem. Phys. Lipids* **2020**, *231*, 104919. [[CrossRef](#)] [[PubMed](#)]
72. Kulig, W.; Korolainen, H.; Zatorska, M.; Kwolek, U.; Wydro, P.; Kepczynski, M.; Róg, T. Complex behavior of phosphatidylcholine-phosphatidic acid bilayers and monolayers: Effect of acyl chain unsaturation. *Langmuir* **2019**, *35*, 5944–5956. [[CrossRef](#)] [[PubMed](#)]
73. Santos, H.A.F.; Vila-Viçosa, D.; Teixeira, V.H.; Baptista, A.M.; Machuqueiro, M. Constant-pH MD simulations of DMPA/DMPC lipid bilayers. *J. Chem. Theory Comput.* **2015**, *11*, 5973–5979. [[CrossRef](#)] [[PubMed](#)]
74. Tanguy, E.; Kassas, N.; Vitale, N. Protein-phospholipid interaction motifs: A focus on phosphatidic acid. *Biomolecules* **2018**, *8*, 20. [[CrossRef](#)]
75. Cao, C.; Wang, P.; Song, H.; Jing, W.; Shen, L.; Zhang, Q.; Zhang, W. Phosphatidic acid binds to and regulates guanine nucleotide exchange factor 8 (GEF8) activity in *Arabidopsis*. *Funct. Plant Biol.* **2017**, *44*, 1029–1038. [[CrossRef](#)]
76. Cools, T.L.; Vriens, K.; Struyfs, C.; Verbandt, S.; Ramada, M.H.S.; Brand, G.D.; Bloch, C.; Koch, B.; Traven, A.; Drijfhout, J.W.; et al. The antifungal plant defensin HsAFP1 is a phosphatidic acid-interacting peptide inducing membrane permeabilization. *Front. Microbiol.* **2017**, *8*, 2295. [[CrossRef](#)]
77. Im, Y.J.; Davis, A.J.; Perera, I.Y.; Johannes, E.; Allen, N.S.; Boss, W.F. The N-terminal membrane occupation and recognition nexus domain of *Arabidopsis* phosphatidylinositol phosphate kinase 1 regulates enzyme activity. *J. Biol. Chem.* **2007**, *282*, 5443–5452. [[CrossRef](#)]
78. Petersen, J.; Eriksson, S.K.; Harryson, P.; Pierog, S.; Colby, T.; Bartels, D.; Röhrig, H. The lysine-rich motif of intrinsically disordered stress protein CDeT11-24 from *Craterostigma plantagineum* is responsible for phosphatidic acid binding and protection of enzymes from damaging effects caused by desiccation. *J. Exp. Bot.* **2012**, *63*, 4919–4929. [[CrossRef](#)]
79. Wang, P.; Shen, L.; Guo, J.; Jing, W.; Qu, Y.; Li, W.; Bi, R.; Xuan, W.; Zhang, Q.; Zhang, W. Phosphatidic acid directly regulates PINOID-dependent phosphorylation and activation of the PIN-FORMED2 auxin efflux transporter in response to salt stress. *Plant Cell* **2019**, *31*, 250–271. [[CrossRef](#)]
80. Julkowska, M.M.; McLoughlin, F.; Galvan-Ampudia, C.S.; Rankenberg, J.M.; Kawa, D.; Klimecka, M.; Haring, M.A.; Munnik, T.; Kooijman, E.E.; Testerink, C. Identification and functional characterization of the *Arabidopsis* Snf1-related protein kinase SnRK2.4 phosphatidic acid-binding domain. *Plant Cell Environ.* **2015**, *38*, 614–624. [[CrossRef](#)]
81. Shen, L.; Tian, Q.; Yang, L.; Zhang, H.; Shi, Y.; Shen, Y.; Zhou, Z.; Wu, Q.; Zhang, Q.; Zhang, W. Phosphatidic acid directly binds with rice potassium channel OsAKT2 to inhibit its activity. *Plant J.* **2020**, *102*, 649–665. [[CrossRef](#)] [[PubMed](#)]
82. Shen, L.; Yang, L.; Zhang, W. Multiple basic amino acid residues contribute to phosphatidic acid-mediated inhibition of rice potassium channel OsAKT2. *Plant Signal. Behav.* **2020**, *15*, 1789818. [[CrossRef](#)] [[PubMed](#)]
83. Yperman, K.; Papageorgiou, A.C.; Merceron, R.; De Munck, S.; Bloch, Y.; Eeckhout, D.; Jiang, Q.; Tack, P.; Grigoryan, R.; Evangelidis, T.; et al. Distinct EH domains of the endocytic TPLATE complex confer lipid and protein binding. *Nat. Commun.* **2021**, *12*, 3050. [[CrossRef](#)] [[PubMed](#)]
84. Testerink, C.; Larsen, P.B.; van der Does, D.; van Himbergen, J.A.J.; Munnik, T. Phosphatidic acid binds to and inhibits the activity of *Arabidopsis* CTR1. *J. Exp. Bot.* **2007**, *58*, 3905–3914. [[CrossRef](#)] [[PubMed](#)]
85. Pleskot, R.; Pejchar, P.; Žárský, V.; Staiger, C.J.; Potocký, M. Structural insights into the inhibition of actin-capping protein by interactions with phosphatidic acid and phosphatidylinositol (4,5)-bisphosphate. *PLoS Comput. Biol.* **2012**, *8*, e1002765. [[CrossRef](#)] [[PubMed](#)]

86. Pandit, S.; Dalal, V.; Mishra, G. Identification of novel phosphatidic acid binding domain on sphingosine kinase 1 of *Arabidopsis thaliana*. *Plant Physiol. Biochem.* **2018**, *128*, 178–184. [[CrossRef](#)]
87. Zimmerberg, J.; Kozlov, M.M. How proteins produce cellular membrane curvature. *Nat. Rev. Mol. Cell Biol.* **2005**, *7*, 9–19. [[CrossRef](#)]
88. Yao, H.; Wang, G.; Guo, L.; Wang, X. Phosphatidic acid interacts with a MYB transcription factor and regulates its nuclear localization and function in *Arabidopsis*. *Plant Cell* **2013**, *25*, 5030–5042. [[CrossRef](#)]
89. Kim, S.-C.; Guo, L.; Wang, X. Phosphatidic acid binds to cytosolic glyceraldehyde-3-phosphate dehydrogenase and promotes its cleavage in *Arabidopsis*. *J. Biol. Chem.* **2013**, *288*, 11834–11844. [[CrossRef](#)]
90. Gautier, R.; Douguet, D.; Antony, B.; Drin, G. HELIQUEST: A web server to screen sequences with specific α -helical properties. *Bioinformatics* **2008**, *24*, 2101–2102. [[CrossRef](#)]
91. Roy Choudhury, S.; Pandey, S. Phosphatidic acid binding inhibits RGS1 activity to affect specific signaling pathways in *Arabidopsis*. *Plant J.* **2017**, *90*, 466–477. [[CrossRef](#)] [[PubMed](#)]
92. Kim, S.-C.; Nusinow, D.A.; Sorkin, M.L.; Prunedo-Paz, J.; Wang, X. Interaction and regulation between lipid mediator phosphatidic acid and circadian clock regulators. *Plant Cell* **2019**, *31*, 399–416. [[CrossRef](#)] [[PubMed](#)]
93. Qu, Y.; Song, P.; Hu, Y.; Jin, X.; Jia, Q.; Zhang, X.; Chen, L.; Zhang, Q. Regulation of stomatal movement by cortical microtubule organization in response to darkness and ABA signaling in *Arabidopsis*. *Plant Growth Regul.* **2018**, *84*, 467–479. [[CrossRef](#)]
94. Shen, L.; Zhuang, B.; Wu, Q.; Zhang, H.; Nie, J.; Jing, W.; Yang, L.; Zhang, W. Phosphatidic acid promotes the activation and plasma membrane localization of MKK7 and MKK9 in response to salt stress. *Plant Sci.* **2019**, *287*, 110190. [[CrossRef](#)]
95. Zhou, Y.; Zhou, D.M.; Yu, W.W.; Shi, L.L.; Zhang, Y.; Lai, Y.X.; Huang, L.P.; Qi, H.; Chen, Q.F.; Yao, N.; et al. Phosphatidic acid modulates MPK3- and MPK6-mediated hypoxia signaling in *Arabidopsis*. *Plant Cell* **2021**, *34*, 889–909. [[CrossRef](#)]
96. Barbosa, I.C.R.; Shikata, H.; Zourelidou, M.; Heilmann, M.; Heilmann, I.; Schwechheimer, C. Phospholipid composition and a polybasic motif determine D6 PROTEIN KINASE polar association with the plasma membrane and tropic responses. *Development* **2016**, *143*, 4687–4700. [[CrossRef](#)]
97. Klimecka, M.; Bucholc, M.; Maszkowska, J.; Krzywińska, E.; Goch, G.; Lichocka, M.; Szczegielniak, J.; Dobrowolska, G. Regulation of ABA-non-activated SNF1-related protein kinase 2 signaling pathways by phosphatidic acid. *Int. J. Mol. Sci.* **2020**, *21*, 4984. [[CrossRef](#)]
98. Chen, X.; Li, L.; Xu, B.; Zhao, S.; Lu, P.; He, Y.; Ye, T.; Feng, Y.-Q.; Wu, Y. Phosphatidylinositol-specific phospholipase C2 functions in auxin-modulated root development. *Plant Cell Environ.* **2018**, *42*, 1441–1457. [[CrossRef](#)]
99. Peters, C.; Kim, S.-C.; Devaiah, S.; Li, M.; Wang, X. Non-specific phospholipase C5 and diacylglycerol promote lateral root development under mild salt stress in *Arabidopsis*. *Plant Cell Environ.* **2014**, *37*, 2002–2013. [[CrossRef](#)]
100. Tan, S.; Zhang, X.; Kong, W.; Yang, X.-L.; Molnár, G.; Vondráková, Z.; Filepová, R.; Petrášek, J.; Friml, J.; Xue, H.-W. The lipid code-dependent phosphoswitch PDK1–D6PK activates PIN-mediated auxin efflux in *Arabidopsis*. *Nat. Plants* **2020**, *6*, 556–569. [[CrossRef](#)]
101. Chang, X.; Riemann, M.; Liu, Q.; Nick, P. Actin as Deathly Switch? How Auxin Can Suppress Cell-Death Related Defence. *PLoS ONE* **2015**, *10*, e0125498. [[CrossRef](#)] [[PubMed](#)]
102. Altúzar-Molina, A.R.; Muñoz-Sánchez, J.A.; Vázquez-Flota, F.; Monforte-González, M.; Racagni-Di Palma, G.; Hernández-Sotomayor, S.M.T. Phospholipidic signaling and vanillin production in response to salicylic acid and methyl jasmonate in *Capsicum chinense* J. cells. *Plant Physiol. Biochem.* **2011**, *49*, 151–158. [[CrossRef](#)] [[PubMed](#)]
103. Kalachova, T.; Iakovenko, O.; Kretinin, S.; Kravets, V. Involvement of phospholipase D and NADPH-oxidase in salicylic acid signaling cascade. *Plant Physiol. Biochem.* **2013**, *66*, 127–133. [[CrossRef](#)] [[PubMed](#)]
104. Rodas-Junco, B.A.; Muñoz-Sánchez, J.A.; Vázquez-Flota, F.; Hernández-Sotomayor, S.M.T. Salicylic-acid elicited phospholipase D responses in *Capsicum chinense* cell cultures. *Plant Physiol. Biochem.* **2015**, *90*, 32–37. [[CrossRef](#)]
105. Krinke, O.; Flemr, M.; Vergnolle, C.; Collin, S.; Renou, J.-P.; Taconnat, L.; Yu, A.; Burketová, L.; Valentová, O.; Zachowski, A.; et al. Phospholipase D Activation Is an Early Component of the Salicylic Acid Signaling Pathway in *Arabidopsis* Cell Suspensions. *Plant Physiol.* **2009**, *150*, 424. [[CrossRef](#)]
106. Rainteau, D.; Humbert, L.; Delage, E.; Vergnolle, C.; Cantrel, C.; Maubert, M.-A.; Lanfranchi, S.; Maldiney, R.; Collin, S.; Wolf, C.; et al. Acyl chains of phospholipase D transphosphatidylation products in *Arabidopsis* cells: A study using multiple reaction monitoring mass spectrometry. *PLoS ONE* **2012**, *7*, e41985. [[CrossRef](#)]
107. Kalachova, T.; Puga-Freitas, R.; Kravets, V.; Soubigou-Taconnat, L.; Repellin, A.; Balzergue, S.; Zachowski, A.; Ruelland, E. The inhibition of basal phosphoinositide-dependent phospholipase C activity in *Arabidopsis* suspension cells by abscisic or salicylic acid acts as a signalling hub accounting for an important overlap in transcriptome remodelling induced by these hormones. *Env. Exp. Bot.* **2016**, *123*, 37–49. [[CrossRef](#)]
108. Ruelland, E.; Pokotylo, I.; Djafi, N.; Cantrel, C.; Repellin, A.; Zachowski, A. Salicylic acid modulates levels of phosphoinositide dependent-phospholipase C substrates and products to remodel the *Arabidopsis* suspension cell transcriptome. *Front. Plant Sci* **2014**, *5*, 608. [[CrossRef](#)]
109. Rodas-Junco, B.A.; Cab-Guillen, Y.; Muñoz-Sánchez, J.A.; Vázquez-Flota, F.; Monforte-Gonzalez, M.; Hernández-Sotomayor, S.M.T. Salicylic acid induces vanillin synthesis through the phospholipid signaling pathway in *Capsicum chinense* cell cultures. *Plant Signal. Behav.* **2013**, *8*, e26752. [[CrossRef](#)]

110. Wimalasekera, R.; Pejchar, P.; Holk, A.; Martinec, J.; Scherer, G.F.E. Plant phosphatidylcholine-hydrolyzing phospholipases C NPC3 and NPC4 with roles in root development and brassinolide signaling in *Arabidopsis thaliana*. *Mol. Plant* **2010**, *3*, 610–625. [[CrossRef](#)]
111. Pokotylo, I.V.; Kretynin, S.V.; Khripach, V.A.; Ruelland, E.; Blume, Y.B.; Kravets, V.S. Influence of 24-epibrassinolide on lipid signalling and metabolism in *Brassica napus*. *Plant Growth Regul.* **2014**, *73*, 9–17. [[CrossRef](#)]
112. Derevyanchuk, M.; Kretynin, S.; Iakovenko, O.; Litvinovskaya, R.; Zhabinskii, V.; Martinec, J.; Blume, Y.; Khripach, V.; Kravets, V. Effect of 24-epibrassinolide on *Brassica napus* alternative respiratory pathway, guard cells movements and phospholipid signaling under salt stress. *Steroids* **2017**, *117*, 16–24. [[CrossRef](#)] [[PubMed](#)]
113. Derevyanchuk, M.; Kretynin, S.; Kolesnikov, Y.; Litvinovskaya, R.; Martinec, J.; Khripach, V.; Kravets, V. Seed germination, respiratory processes and phosphatidic acid accumulation in *Arabidopsis* diacylglycerol kinase knockouts – The effect of brassinosteroid, brassinazole and salinity. *Steroids* **2019**, *147*, 28–36. [[CrossRef](#)] [[PubMed](#)]
114. Kumar, D.; Kumar, R.; Baek, D.; Hyun, T.-K.; Chung, W.S.; Yun, D.-J.; Kim, J.-Y. *Arabidopsis thaliana* RECEPTOR DEAD KINASE1 functions as a positive regulator in plant responses to ABA. *Mol. Plant* **2017**, *10*, 223–243. [[CrossRef](#)]
115. Jiang, Y.; Wu, K.; Lin, F.; Qu, Y.; Liu, X.; Zhang, Q. Phosphatidic acid integrates calcium signaling and microtubule dynamics into regulating ABA-induced stomatal closure in *Arabidopsis*. *Planta* **2014**, *239*, 565–575. [[CrossRef](#)]
116. Uwase, G.; Enrico, T.; Chelimo, D.; Keyser, B.; Johnson, R. Measuring gene expression in bombarded barley aleurone layers with increased throughput. *J. Vis. Exp.* **2018**, *133*, 56728. [[CrossRef](#)]
117. Pandey, S.; Wang, R.-S.; Wilson, L.; Li, S.; Zhao, Z.; Gookin, T.E.; Assmann, S.M.; Albert, R. Boolean modeling of transcriptome data reveals novel modes of heterotrimeric G-protein action. *Mol. Syst. Biol.* **2010**, *6*, 372. [[CrossRef](#)]
118. Bates, G.W.; Rosenthal, D.M.; Sun, J.; Chattopadhyay, M.; Peffer, E.; Yang, J.; Ort, D.R.; Jones, A.M. A comparative study of the *Arabidopsis thaliana* guard-cell transcriptome and its modulation by sucrose. *PLoS ONE* **2012**, *7*, e49641. [[CrossRef](#)]
119. Cenzano, A.; Cantoro, R.; Racagni, G.; De Los Santos-Briones, C.; Hernández-Sotomayor, T.; Abdala, G. Phospholipid and phospholipase changes by jasmonic acid during stolon to tuber transition of potato. *Plant Growth Regul.* **2008**, *56*, 307. [[CrossRef](#)]
120. Profotová, B.; Burketová, L.; Novotná, Z.; Martinec, J.; Valentová, O. Involvement of phospholipases C and D in early response to SAR and ISR inducers in *Brassica napus* plants. *Plant Physiol. Biochem.* **2006**, *44*, 143–151. [[CrossRef](#)]
121. Muñoz-Sánchez, J.A.; Altúzar-Molina, A.; Hernández-Sotomayor, S.M.T. Phospholipase signaling is modified differentially by phyto regulators in *Capsicum chinense* J. cells. *Plant Signal. Behav.* **2012**, *7*, 1103–1105. [[CrossRef](#)] [[PubMed](#)]
122. Yang, D.; Liu, X.; Yin, X.; Dong, T.; Yu, M.; Wu, Y. Rice non-specific phospholipase C6 is involved in mesocotyl elongation. *Plant Cell Physiol.* **2021**, *62*, 985–1000. [[CrossRef](#)] [[PubMed](#)]
123. Amini, A.; Glévairec, G.; Andreu, F.; Reverdiau, P.; Rideau, M.; Crèche, J. Effects of phosphatidic acid on cytokinin signal transduction in periwinkle cells. *J. Plant Growth Regul.* **2008**, *27*, 394–399. [[CrossRef](#)]
124. Kravets, V.S.; Kolesnikov, Y.S.; Kretynin, S.V.; Getman, I.A.; Romanov, G.A. Rapid activation of specific phospholipase(s) D by cytokinin in *Amaranthus* assay system. *Physiol. Plant* **2010**, *138*, 249–255. [[CrossRef](#)]
125. Qu, Y.; An, Z.; Zhuang, B.; Jing, W.; Zhang, Q.; Zhang, W. Copper amine oxidase and phospholipase D act independently in abscisic acid (ABA)-induced stomatal closure in *Vicia faba* and *Arabidopsis*. *J. Plant Res.* **2014**, *127*, 533–544. [[CrossRef](#)]
126. Echevarría-Machado, I.; Ramos-Díaz, A.; Brito-Argáez, L.; Racagni-Di Palma, G.; Loyola-Vargas, V.M.; Hernández-Sotomayor, S.M.T. Polyamines modify the components of phospholipids-based signal transduction pathway in *Coffea arabica* L. cells. *Plant Physiol. Biochem.* **2005**, *43*, 874–881. [[CrossRef](#)]
127. Gully, K.; Pelletier, S.; Guillou, M.-C.; Ferrand, M.; Aligon, S.; Pokotylo, I.; Perrin, A.; Vergne, E.; Fagard, M.; Ruelland, E.; et al. The SCOOP12 peptide regulates defense response and root elongation in *Arabidopsis thaliana*. *J. Exp. Bot.* **2019**, *70*, 1349–1365. [[CrossRef](#)]
128. Jones, A.M.; Xuan, Y.; Xu, M.; Wang, R.-S.; Ho, C.-H.; Lalonde, S.; You, C.H.; Sardi, M.I.; Parsa, S.A.; Smith-Valle, E.; et al. Border control—a membrane-linked interactome of *Arabidopsis*. *Science* **2014**, *344*, 711–716. [[CrossRef](#)]
129. Scuffi, D.; Nietzel, T. Hydrogen sulfide increases production of NADPH oxidase-dependent hydrogen peroxide and phospholipase D-derived phosphatidic acid in guard cell signaling. *Plant Physiol.* **2018**, *176*, 2532–2542. [[CrossRef](#)]
130. Chen, J.; Wang, P.; de Graaf, B.H.J.; Zhang, H.; Jiao, H.; Tang, C.; Zhang, S.; Wu, J. Phosphatidic acid counteracts S-RNase signaling in pollen by stabilizing the actin cytoskeleton. *Plant Cell* **2018**, *30*, 1023–1039. [[CrossRef](#)]
131. Chen, Z.-F.; Ru, J.-N.; Sun, G.-Z.; Du, Y.; Chen, J.; Zhou, Y.-B.; Chen, M.; Ma, Y.-Z.; Xu, Z.-S.; Zhang, X.-H. Genomic-wide analysis of the PLC family and detection of GmPI-PLC7 responses to drought and salt stresses in soybean. *Front. Plant Sci.* **2021**, *12*, 631470. [[CrossRef](#)] [[PubMed](#)]
132. Robert-Seilaniantz, A.; MacLean, D.; Jikumaru, Y.; Hill, L.; Yamaguchi, S.; Kamiya, Y.; Jones, J.D.G. The microRNA miR393 re-directs secondary metabolite biosynthesis away from camalexin and towards glucosinolates. *Plant J.* **2011**, *67*, 218–231. [[CrossRef](#)] [[PubMed](#)]
133. Goda, H.; Sasaki, E.; Akiyama, K.; Maruyama-Nakashita, A.; Nakabayashi, K.; Li, W.; Ogawa, M.; Yamauchi, Y.; Preston, J.; Aoki, K.; et al. The AtGenExpress hormone and chemical treatment data set: Experimental design, data evaluation, model data analysis and data access. *Plant J. Cell Mol. Biol.* **2008**, *55*, 526–542. [[CrossRef](#)]
134. Van Zanten, M.; Basten Snoek, L.; Van Eck-Stouten, E.; Proveniers, M.C.G.; Torii, K.U.; Voesenek, L.A.C.J.; Peeters, A.J.M.; Millenaar, F.F. Ethylene-induced hyponastic growth in *Arabidopsis thaliana* is controlled by ERECTA. *Plant J.* **2010**, *61*, 83–95. [[CrossRef](#)]

135. Argyros, R.D.; Mathews, D.E.; Chiang, Y.-H.; Palmer, C.M.; Thibault, D.M.; Etheridge, N.; Argyros, D.A.; Mason, M.G.; Kieber, J.J.; Schaller, G.E. Type B Response Regulators of *Arabidopsis* play key roles in cytokinin signaling and plant development. *Plant Cell* **2008**, *20*, 2102–2116. [[CrossRef](#)]
136. Gupta, A.; Singh, M.; Laxmi, A. Multiple interactions between glucose and brassinosteroid signal transduction pathways in *Arabidopsis* are uncovered by whole-genome transcriptional profiling. *Plant Physiol.* **2015**, *168*, 1091–1105. [[CrossRef](#)]
137. Mueller, S.; Hilbert, B.; Dueckershoff, K.; Roitsch, T.; Krischke, M.; Mueller, M.J.; Berger, S. General detoxification and stress responses are mediated by oxidized lipids through TGA transcription factors in *Arab.* *Plant Cell* **2008**, *20*, 768–785. [[CrossRef](#)]
138. Haruta, M.; Sabat, G.; Stecker, K.; Minkoff, B.B.; Sussman, M.R. A peptide hormone and its receptor protein kinase regulate plant cell expansion. *Science* **2014**, *343*, 408–411. [[CrossRef](#)]
139. Mashiguchi, K.; Sakaki, E.; Shimada, Y.; Nagae, M.; Ueno, K.; Nakano, T.; Yoneyama, K.; Suzuki, Y.; Asami, T. Feedback-regulation of strigolactone biosynthetic genes and strigolactone-regulated genes in *Arabidopsis*. *Biosci. Biotechnol. Biochem.* **2009**, *73*, 2460–2465. [[CrossRef](#)]
140. Welti, R.; Li, W.; Li, M.; Sang, Y.; Biesiada, H.; Zhou, H.E.; Rajashekar, C.B.; Williams, T.D.; Wang, X. Profiling membrane lipids in plant stress responses. Role of phospholipase D alpha in freezing-induced lipid changes in *Arabidopsis*. *J. Biol. Chem.* **2002**, *277*, 31994–32002. [[CrossRef](#)]
141. Noack, L.C.; Bayle, V.; Armengot, L.; Rozier, F.d.r.; Mamode-Cassim, A.; Stevens, F.D.; Caillaud, M.-C.c.; Munnik, T.; Mongrand, S.b.; Pleskot, R.; et al. A nanodomain-anchored scaffolding complex is required for the function and localization of phosphatidylinositol 4-kinase alpha in plants. *Plant Cell* **2021**, *34*, 302–332. [[CrossRef](#)] [[PubMed](#)]
142. Mamode Cassim, A.; Gouguet, P.; Gronnier, J.; Laurent, N.; Germain, V.; Grison, M.; Boutté, Y.; Gerbeau-Pissot, P.; Simon-Plas, F.; Mongrand, S. Plant lipids: Key players of plasma membrane organization and function. *Prog. Lipid Res.* **2019**, *73*, 1–27. [[CrossRef](#)] [[PubMed](#)]
143. Wang, T.; Hu, M.; Yuan, D.; Yun, Z.; Gao, Z.; Su, Z.; Zhang, Z. Melatonin alleviates pericarp browning in litchi fruit by regulating membrane lipid and energy metabolisms. *Postharvest Biol. Technol.* **2020**, *160*, 111066. [[CrossRef](#)]
144. Wong, A.; Donaldson, L.; Portes, M.T.; Eppinger, J.; Feijó, J.A.; Gehring, C. *Arabidopsis* DIACYLGLYCEROL KINASE4 is involved in nitric oxide-dependent pollen tube guidance and fertilization. *Development* **2020**, *147*. [[CrossRef](#)]
145. Yao, S.; Peng, S.; Wang, X. Phospholipase Dε interacts with autophagy-related-protein 8 and promotes autophagy in *Arabidopsis* response to nitrogen deficiency. *Plant J.* **2022**. [[CrossRef](#)]
146. Holland, P.; Knævelsrud, H.; Søreng, K.; Mathai, B.J.; Lystad, A.H.; Pankiv, S.; Bjørndal, G.T.; Schultz, S.W.; Lobert, V.H.; Chan, R.B.; et al. HS1BP3 negatively regulates autophagy by modulation of phosphatidic acid levels. *Nat. Commun.* **2016**, *7*, 13889. [[CrossRef](#)]
147. Su, Y.; Li, M.; Guo, L.; Wang, X. Different effects of phospholipase Dζ2 and non-specific phospholipase C4 on lipid remodeling and root hair growth in *Arabidopsis* response to phosphate deficiency. *Plant J. Cell Mol. Biol.* **2018**, *94*, 315–326. [[CrossRef](#)]
148. Wang, Y.; Duran, H.G.S.; van Haarst, J.C.; Schijlen, E.G.W.M.; Ruyter-Spira, C.; Medema, M.H.; Dong, L.; Bouwmeester, H.J. The role of strigolactones in P deficiency induced transcriptional changes in tomato roots. *BMC Plant Biol.* **2021**, *21*, 349. [[CrossRef](#)]
149. Takáč, T.; Šamajová, O.; Vadovič, P.; Pechan, T.; Šamaj, J. Shot-gun proteomic analysis on roots of *Arabidopsis pldα1* mutants suggesting the involvement of PLDα1 in mitochondrial protein import, vesicular trafficking and glucosinolate biosynthesis. *Int. J. Mol. Sci.* **2018**, *20*, 82. [[CrossRef](#)]
150. Takáč, T.; Pechan, T.; Šamajová, O.; Šamaj, J. Proteomic analysis of *Arabidopsis pldα1* mutants revealed an important role of phospholipase D alpha 1 in chloroplast biogenesis. *Front. Plant Sci.* **2019**, *10*, 89. [[CrossRef](#)]