

REVIEW

UDC 577.152.271:577.152.311:577.171.55:577.125.2:581.192.7

doi: <https://doi.org/10.15407/ubj96.01.005>PHOSPHATIDIC ACID FORMATION
AND SIGNALING IN PLANT CELLS

Y. S. KOLESNIKOV, S. V. KRETYNIN, V. S. KRAVETS, Y. K. BUKHONSKA✉

V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry,
National Academy of Sciences of Ukraine, Kyiv;
✉e-mail: yasya.yaroslavka@gmail.com**Received:** 07 November 2023; **Revised:** 18 December 2023; **Accepted:** 01 February 2024

This review contains updated information on the structure, localization and regulation of phosphatidic acid (PA)-producing enzymes phospholipase D, phosphoinositide-specific and non-specific phospholipases C and diacylglycerol kinases is analyzed. The specific role of PA and PA-producing enzymes in plant stress signaling is discussed.

Key words: *phosphatidic acid, plant cells, phospholipases D and C, diacylglycerol kinase, phosphatidic acid-binding proteins, plant stress signaling.*

Membranes are composed of various types of phospholipids, and their role extends beyond structural to include informational functions. The latter involves membrane phospholipids serving as precursors to intracellular signaling molecules. One extensively studied informational function is their role as second messengers in intracellular signaling pathways. Phosphatidic acids (PA) are widely recognized as phospholipid second messengers that translate extracellular information, such as hormonal, stress, and developmental signals, into specific cellular responses. They play a crucial role in modulating cellular metabolism to maintain a balance in plant stress tolerance, growth, and development [1-3].

Phosphatidic acid is a minor membrane phospholipid containing phosphoryl glycerol with two fatty acid chains. Signaling phosphatidic acid is generated through the activation of phospholipases D, which cleave structural membrane phospholipids (e.g., phosphatidylcholine). This cleavage results in the production of phosphatidic acid and a free head group (e.g., choline) [2]. Additionally, PA can be produced through the phosphorylation of diacylglycerol (DAG), a process catalyzed by diacylglycerol kinase [3] (Fig. 1).

In response to stress and hormone action, PA level in cells undergoes rapid modification [4]. Once formed in response to extracellular stimuli within membranes, PA binds to specific proteins that regulate downstream responses crucial for its function in the regulation of growth, development, and stress responses. For example, PA inhibits autophagy by binding to GAPC or PGK3 proteins [5]. Additionally, PA binds to *Arabidopsis* arginase ARGH2, stimulating its activity [6]. Given that PA is present at high levels in all cells under basal conditions, it was initially unclear how PA achieves signaling specificity in response to various extracellular actions. Possible determinants of PA signaling specificity in plants are listed below.

**Recent developments on phospholipid
signaling enzymes involved
in PA production****Advances in defining the functions,
regulation, and localization
of phospholipase D isoforms**

Structure and localization of plant PLDs. Phospholipase D (PLD) is the enzyme that hydrolyzes structural membrane phospholipids, directly producing phosphatidic acid. For instance, rice

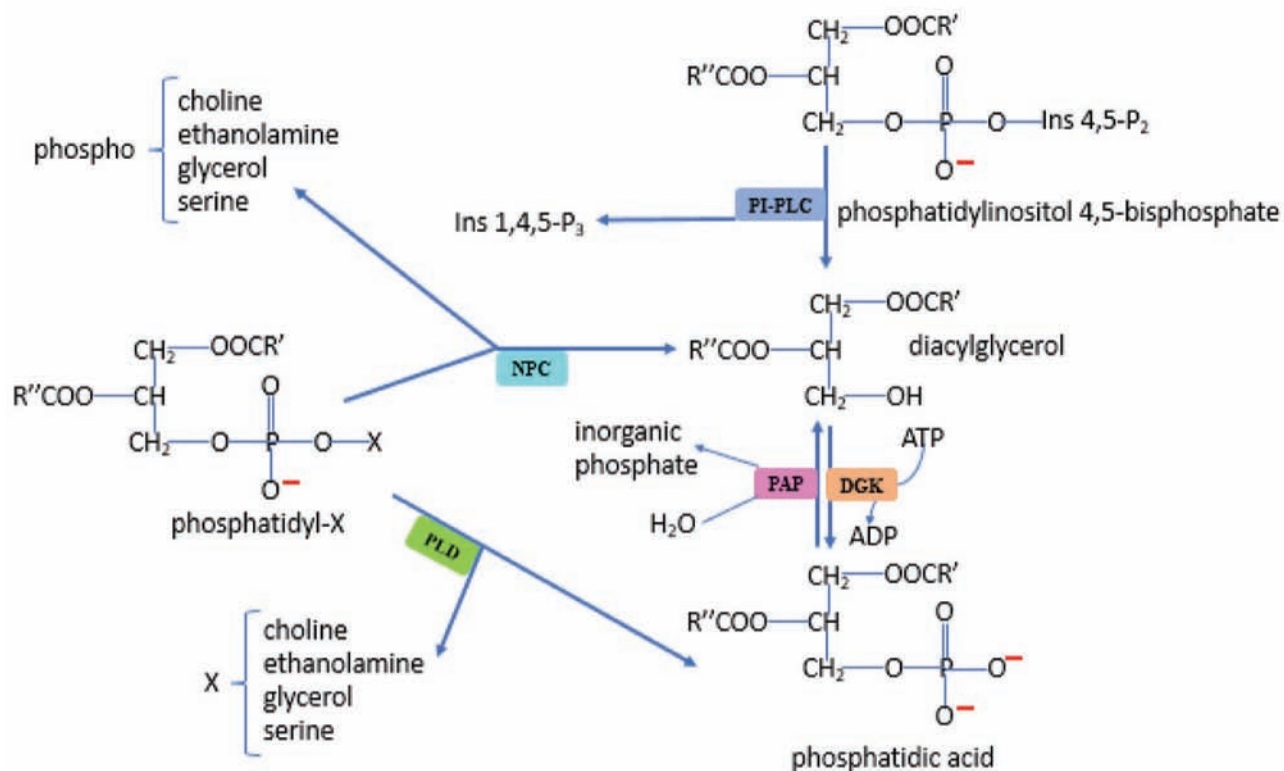


Fig. 1. Main pathways of phosphatidic acid metabolism in plants. DGK – diacylglycerol kinase, Ins – inositol, NPC – non-specific phospholipase C, PAP – phosphatidic acid phosphatase, PI-PLC – phosphoinositide-specific phospholipase C, PLD – phospholipase D

PLD α 6 can hydrolyze various substrates, including phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylserine [7]. PLD activity, acting on glycosylinositol phosphoceramide, has also been detected in plants [8]. Additionally, the PLD γ isoform generates N-acylethanolamines together with PA in response to infection [9].

PLDs in plants are represented by various isoforms (α , β , γ , δ , ϵ , ζ , κ , and ϕ) [7, 10, 11] each possessing a typical domain structure (Fig. 2). In peanut, for example, isoforms α , β , γ , δ , and ϵ belong to the C2-PLDs, containing the calcium/phospholipid-binding C2 domain at the N-terminal. PLD ζ s containing PX and/or PH domains at the N-terminal are classified as PX/PH-PLDs. PLD ϕ s contain a signal peptide at the N-terminal instead of the C2 or PX/PH domain, placing them in the SP-PLDs category [10]. Similarly, in rice, PLD α s, PLD β s, PLD δ s, and PLD κ contain the C2 domain, while PLD ζ s have the PX and PH domains. PLD ϕ in rice has a signal peptide at the N-terminus. All rice C2-PLDs and PX/PH-PLDs possess two HKD (HxKxxxxD) catalytic motifs,

except PLD α 7, which has a mutation (RxKxxxxD) in the second HKD motif [7]. Notably, PLD γ s isoforms were identified in *Arabidopsis* but not in rice, while PLD κ and PLD ϕ were found in rice but not in *Arabidopsis* [7]. Evolutionarily, PLD γ , PLD κ , and PLD ϕ were suggested to be duplicated later, as they are not found in lower plants and differ between monocot and dicot plants. In contrast, PLD α s and PLD δ s are found in both lower and higher plant species, indicating their original and conserved nature among plant species [7]. All sorghum PLD family members harbor two conserved domains (HKD1 and 2) with catalytic activity, with most members containing a C2 domain. In the zeta subfamily, the C2 domain is replaced by the PX and PH domains [12]. Conservation of two HKD (HxKxxxxD) domains is found in all PLD genes of both jute species, except for CoPLD δ -2, which has only one HKD domain [13]. In alfalfa, two HKD structural domains are highly conserved, with some exceptions such as the mutation of D to K in the second HKD structural domain of MsPLD03 and the deletion of D in the second HKD structural domain of MsPLD56 [14].

Except for VviPLD ϕ , which is the only grapevine SP-PLD, all grapevine PLDs have a PIP₂-binding motif [15]. In peanut PLD, this motif is represented by the sequence “xxGPRxPWHDXHxxxxGPAxxD-VLTNFE $\times$ RWRK $\times$ Gx” [10]. Additionally, trans-membrane helices were predicted in some pineapple PLDs [16]. The tobacco PLD δ tertiary structure consists of a tightly packed globular catalytic domain with an attached C-terminal domain and a somewhat loosely connected N-terminal C2 domain [17]. Therefore, specific structure determinants within PLD could be involved in regulating the enzyme through interaction with modulators or in modifying its specific localization in cells, thereby affecting the number of PA molecules produced and the strength of the PA signal.

The cellular localization of PLD determines the site where phosphatidic acid is formed upon PLD activation, and this localization can vary within plant cells. For example, most of the alfalfa *MsPLDs* are predicted to be distributed in the cytoplasm, followed by the vacuole, endoplasmic reticulum, and chloroplast [14]. Similarly, the majority of *SbPLDs* are predicted to be in the cytoplasm, with three *SbPLDs* located in the endoplasmic reticulum and only *SbPLD* β 1 in the chloroplast. *SbPLD* α 3 is experimentally supported to be located in the cytoplasm in *Arabidopsis* protoplasts [12]. *C. olitorius* and *C. capsularis* PLD proteins are predicted to be localized in the cytoplasm, followed by the endoplasmic reticulum, with *CcPLD*- β 1 and *CoPLD*- β 1 uniquely found in the nucleus [13]. In peanuts, most PLD proteins are predicted in the cytoplasm, endoplasmic reticulum, and vacuole, with a few in the chloroplast, nucleus, and plasma membrane. *AhPLD* ϕ A/B are predicted to be localized at the plasma membrane [10]. Regarding the grapevine PLD, only two proteins had their cellular locations predicted: the VviPLD α 4 was predicted to be in the mitochondria, and the VviPLD ϕ , possessing a signal peptide, was predicted to be secreted [15]. Experimental evidence indicates multiple subcellular locations of *GmPLD* α 1, including the cytoplasm, cytoskeleton-like structures, and, in part, chloroplasts [27]. Subcellular localization experiments indicated that apple *MdPLD*17 is a membrane protein mainly distributed in the cell membrane [11], while pineapple *AcPLD*2 and *AcPLD*9 [16] and cotton *GhPLD*2 [28] were observed in the plasma membrane when expressed in tobacco leaf epidermal cells. In tobacco pollen tubes, *NtPLD* δ 1 and *NtPLD* δ 2 showed cytoplasmic localization, while all

membrane-bound tobacco PLD δ (3-5) isoforms, with *NtPLD* δ 5 in particular, are attached to the plasma membrane. *NtPLD* δ 3 is only faintly detected at the plasma membrane, exclusively in the subapical zone, while plasma membrane localization of *NtPLD* δ 4-5 was more pronounced and extended further back to the pollen tube shank [17].

Recent studies indicate that the PLD that produces signaling PA seems to be localized at the plasma membrane. PA levels increased by PLD α 1 and PLD δ in response to ammonium application were observed at the plasma membrane in *Arabidopsis* roots [29]. Rapid relocation of *AtPLD* δ to plasma membrane microdomains and its exocytosis in response to pathogen stimuli are involved in plant innate immunity responses [30].

In addition, specific structural determinants mediate PLD localization in cells. For example, PX and PH domains are responsible for membrane localization of *Arabidopsis* PLD ζ 1 and PLD ζ 2, mainly to the trans-Golgi network and post-Golgi compartments [31]. The N-terminus and central catalytic domain (VLREGTEI motif) of *NtPLD* δ 4 are both required for direct interaction with the plasma membrane. The catalytic domain is required, but not sufficient, for plasma membrane localization of *NtPLD* δ 4 [17]. *AtPLD* γ 1 at the plasma membrane associates with BIR2/3 proteins, the negative regulators of pattern-triggered immunity [32]. Therefore, the specific PLD localization within cells, coupled with its regulation at the structure level, plays a crucial role in determining the subcellular localization of PA signaling and its proximity to PA target proteins.

Regulation of plant PLDs. Results of the recent studies indicate that plant PLDs are directly regulated by a range of molecular mechanisms. For example, different PLD-binding proteins are known in plants, representing one level of PLD regulation. Regulator of flowering and stress *BdRFS* protein binds to *BdPLD* α 1, affecting phospholipid metabolism [33]. Rice PLD α 1 decorated microtubules and increased detyrosinated α -tubulin [34]. Low-affinity nitrate transporter NRT1.2 binds to *AtPLD* α 1 at the plasma membrane to positively affect ABA sensitivity during seed germination and seedling development [35]. Potato virus Y transmembrane protein 6K2 recruited *NbPLD* α 1 and PA to the membrane-bound viral replication complex, enhancing the production of *NbPLD* α 1-derived PA [36]. Various protein-protein interactions of peanut PLD were pre-

dicted to be with proteins involved in phospholipid transport, stress, defense, and plant development [10], supporting the important role of this regulatory mechanism in PLD modulations in plants. During allelochemical oridonin-induced stomatal closure in *Arabidopsis*, PLD α 1 acted downstream of the heterotrimeric G-protein GPA1 [37], but the direct interaction of these proteins in these responses remains to be investigated.

PLD is actively modified at the protein level by post-translational modifications. For example, S-glutathionylation of apple tree PLD was found in the adult growth phase [38].

Another important mechanism of PLD regulation is protein phosphorylation. Phosphorylation of PLD δ was found in tomato plants resistant to biotic stress [39]. MPK3 and MPK6 interact with and phosphorylate PLD α 1 and PLD δ , which may contribute to feedback inhibition of PA production under submergence [40]. Also, phosphorylation of PLDs from *Physcomitrella patens* (Phypa_117291, Phypa_163602, Phypa_213846) was found in response to ABA [41, 42]. Changes in phosphorylation level in response to cold were reported for tomato PLD (Solyc08g066800) [43]. MAPK cascade-dependent PLD phosphorylation in cotton was found in response to biotic stress [44]. Moreover, the prediction of protein phosphorylation (on serine, threonine, and tyrosine) of PLD in plants, including *Camelina sativa* and *Brassica napus* PLDs, suggested that PLD- α proteins are less influenced by this post-translational modification compared to other isoforms [45].

Lysine 2-hydroxyisobutyrylation was found in rice PLD in response to infection [46]. PLD S-nitrosylation was found for *Arachis hypogaea* PLD in response to aluminum stress [47]. Changes in lysine acetylation of poplar PLDs were observed during bud dormancy release [48]. ROS-induced cysteine oxidation in *Arabidopsis* PLD δ enhances its binding to calcium, which is involved in microtubule organization, stomatal movement, and thermotolerance [49].

Changes in PLD protein levels or stability represent another mechanism of PLD regulation in plants. PLD protein and PA accumulation in response to EPIP peptide were found in abscission zone cells of lupine flowers [50] suggesting a hormone-induced elevation of PA signaling machinery. Rice PLD α 6 was found to be translocated from the cytosol to the nuclei in response to gibberellin treatment [7]. Accumulation of PLD proteins was observed in the roots and shoots of cowpea plants exposed to drought stress [51] and in groundnuts in response to *A. flavus* infection [52].

Exogenous hexaldehyde modulated PLD protein content in pineapple fruits [16]. MPK3 and MPK6 negatively regulate PLD α 1 protein levels during submergence for feedback inhibition of PA production [40].

Key ions in cells are also well-known regulators of plant PLD enzymatic activity. Purified PLD α 6 displayed Ca²⁺-dependent hydrolysis of phospholipids with the highest activity at the mM levels of Ca²⁺ toward phosphatidylcholine [7]. *Nt*PLD δ s possess a similar ability to bind multiple phospholipids *in vitro*, with a strong preference towards negatively charged phospholipids enriched in the plasma membrane. PA formed by *Nt*PLD δ 3 positively affects *Nt*PLD δ 3 plasma membrane binding via a positive feedback mechanism [17]. PI(4,5)P₂ is another PLD phospholipid effector required for substrate hydrolysis [53]. Taken together, PLD regulation by post-translational modifications, bound proteins, protein stability, and small molecules could modulate the PA signal strength and its velocity. This tight regulation is crucial for the precise interaction of PA with its target proteins.

Advances in defining the functions, regulation, and localization of phosphoinositide-specific phospholipase C

Structure and localization of plant PI-PLCs. PI-PLC hydrolyzes phosphoinositide phospholipids (phosphatidylinositol, phosphatidylinositol 4-phosphate, and phosphatidylinositol 4,5-bisphosphate) generating diacylglycerol and inositol 1,4,5-trisphosphate. For example, rice OsPLC4 hydrolyzed PI, PI4P, and PIP₂ to produce DAG and exhibited a higher hydrolytic activity towards PIP₂ and PI4P than PI [54]. PI-PLC activity acting on glycosylinositol phosphoceramide was also detected in plants [8]. A typical PI-PLC structure is shown in Fig. 2. Tomato PI-PLCs, for example, have 4 domains, namely the EF-hand-like domain, the PLCXc catalytic domain, the PLCYc catalytic domain, and the Ca²⁺/phospholipid-binding C2 domain [18]. Similarly, all the members of the orchid *Pe*PI-PLC, *Dc*PI-PLC, and *As*PI-PLC groups consist of X and Y catalytic domains and the calcium/phospholipid-binding domain [22]. In maize, *Zm*PI-PLCs contained the catalytic PI-PLC-X and PI-PLC-Y domains, the C2 domain, whereas an EF hand-like motif was found only

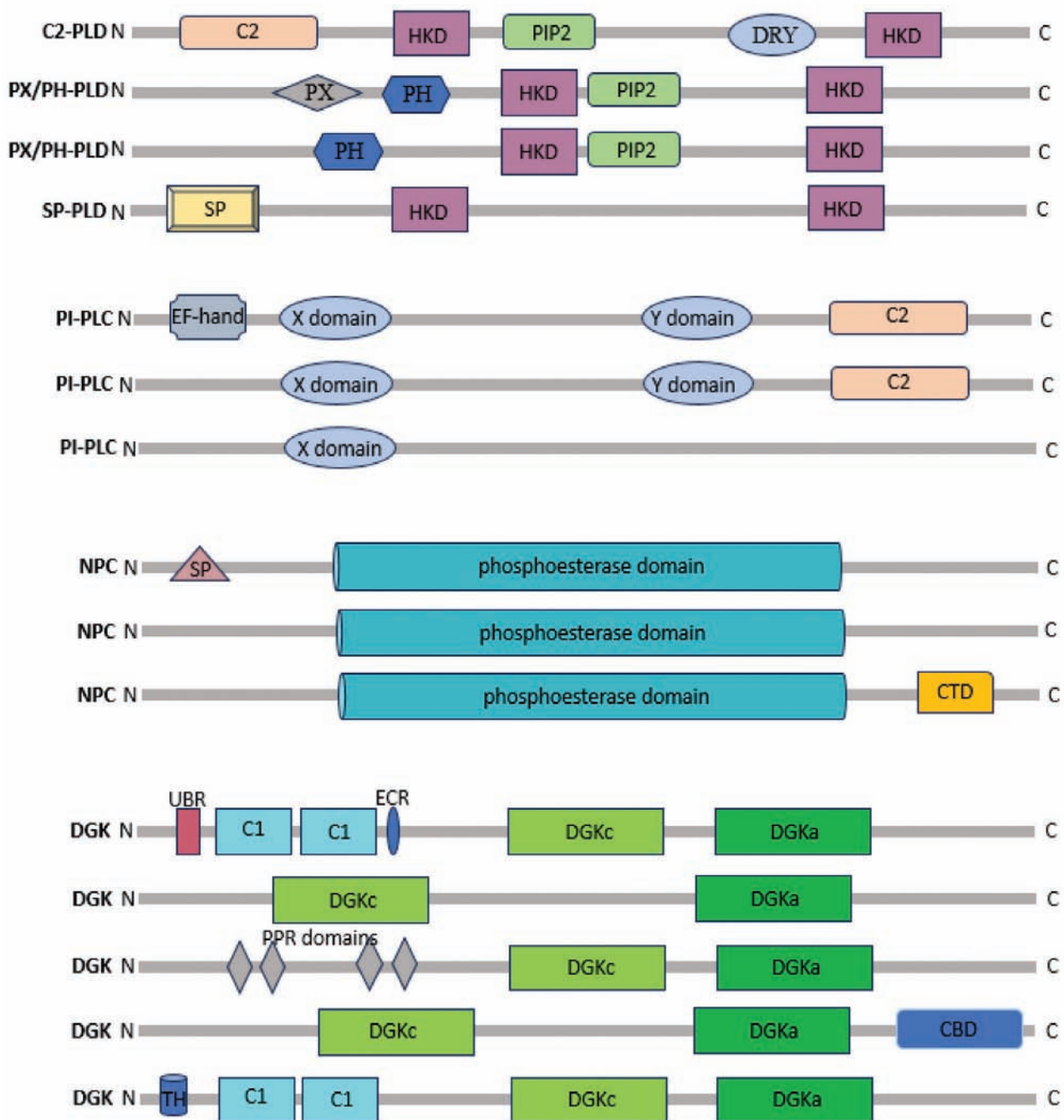


Fig. 2. The structure domains and main functional motifs of all possible types of the protein structures of plant PA-producing enzymes. Data on enzyme structures were taken from the literature [12, 18-26]. CBD – calmodulin-binding domain, CTD – C-terminal domain, C1 – DAG/PE binding motif and transmembrane domains, C2 – calcium/phospholipid-binding domain, DGKa – DGK accessory domain, DGKc – DGK catalytic domain, DRY – G-protein-binding motif, ECR – extended cysteine-rich (extCRD)-like domain, HKD – PLD catalytic domain, PIP2 – phosphoinositide binding region, PPR domain – pentatricopeptide repeat domain, SP – signal peptide, TH – transmembrane helix, UBR – upstream basic region, X and Y domains – PI-PLC catalytic domains

in *ZmPI-PLC4* [19]. In contrast, grapevine PI-PLC presented four characteristic domains: EF-hand, PI-PLC X, PI-PLC Y and the C2 domain, except for *VviPI-PLC4.2*, which does not possess the EF-hand domain [15]. All cotton *GhPIPLCs* possessed four domains, with the exception of *GhPIPLC1A*, *GhPIPLC1D*, and *GhPIPLC6D*, which lacked the EF-hand-like domain [20]. Therefore, specific structure determinants within PI-PLC could be involved in the regulation of the enzyme catalytic activity or interaction with modulators/localization in cells, thus affecting the number of DAG molecules needed for PA production and thereby modulating the activity of the PA signal.

Results of recent investigations in different plants support that PI-PLCs are differentially localized within the cells. For example, *ZmPI-PLCs* were distributed throughout the nucleus and cytoplasm, but *ZmPI-PLC2* was only located in the cytosol [19]. *OsPLC3* was observed in the plasma membrane, cytoplasm, and nucleus of rice cells [55]. Grapevine PI-PLC proteins were predicted to be in the mitochondria (*VviPI-PLC1*, *VviPI-PLC2*, *VviPI-PLC4*, and *VviPI-PLC6*) and in the cytoplasmic membrane (*VviPI-PLC3*, *VviPI-PLC4.2*, *VviPI-PLC5*, and *VviPI-PLC7*) [15]. Chickpea *CaPLC2*, *CaPLC3*, and *CaPLC6* were distributed throughout the cytoplasm, but *CaPLC1*, *CaPLC4*, *CaPLC5*, and *CaNPC1-3* were confirmed to be localized at the plasma membrane [56]. *OsPLC3* was observed in the plasma membrane, cytoplasm, and nucleus of rice protoplasts [55]. Salt-induced cytoplasm-to-plasma membrane translocation of *OsPLC1* was shown in rice [57]. Banana *MaPLC1*, *MaPLC2*, *MaPLC4*, *MbPLC1*, *MbPLC2*, and *MbPLC3* were predicted to be localized to chloroplasts; *MaPLC5*, *MaPLC6*, and *MbPLC4* were localized in mitochondria, and only *MaPLC3* was localized in the cytoplasm. They are not transmembrane proteins [58]. Subcellular localization prediction showed that most of the orchid PI-PLC proteins were cytoplasmic and nuclear [22]. Therefore, specific PI-PLC localization in cells could determine the site of starting DAG production for PA signaling and its vicinity to target proteins.

Mechanisms of PI-PLC regulation. PI-PLC is differently regulated in plant cells. A protein-protein interaction was recently reported for PI-PLC in plants. The interaction between tomato *SIPLC1* and *SIPLC3* may result in the functioning of *SIPLC1* and *SIPLC3* as a dimer, and *SIPLC3* can interact with *SIPLC4*, *SIPLC6*, and other proteins, forming a mul-

timer, but *SIPLC7* does not interact with any *SIPLCs* [18]. Although *Arabidopsis* *PLC1* and G-protein GPA1 mediated the effect of allelochemical cycloas-tragenol on stomatal movements [59], the direct interaction of this PI-PLC with G-protein in regulating these responses was not investigated.

Phosphorylation is an important mechanism of PI-PLC regulation. Cold and cadmium-induced changes in phosphorylation of some PI-PLC were found in tomatoes [43, 60]. Phosphorylation of *PLC3* from *Nicotiana tabacum* was reported in response to the tobacco mosaic virus [61]. Also, MAPK cascade-dependent PLC phosphorylation in cotton was found in response to biotic stress [44]. PLC phosphorylation during fruit development was reported in pepper [62]. In addition, PLC phosphorylation was reported in soybean in response to aluminum stress [63]. *AtPI-PLC2* phosphopeptide abundance was found among significantly upregulated phosphopeptides in plants overexpressing C-TERMINALLY ENCODED PEPTIDE 5 (CEP5) [64]. Lysine crotonylation was found for some PLC proteins in *Camellia sinensis* in response to ammonium [65]. C-terminal proteolysis was found for PI-PLC (Solyc05g052760) from tomato [66].

An adaptor protein, *OsGF14b*, was reported to be an interaction partner of *OsPI-PLC1* that promotes its activity and stability, thereby improving rice salt tolerance [67]. Heterotrimeric G-protein subunits $\beta 1$ and $\alpha 1$ were reported to interact with PI-PLC in *M. truncatula*, but only the G-protein $\alpha 2$ subunit could interact with *P. sativum* *PsPLC* [68]. In addition, an ortholog protein of *Arabidopsis* PI-PLC2 from *C. roseus* (CRO_T004768) was *in silico* predicted by the “CroFGD” database to interact with the peptide receptors CLV1 ortholog (CRO_T007315), HAESA-like 1 ortholog (CRO_T002426), and BARELY ANY MERISTEM 2 ortholog (CRO_T011766) proteins [69].

Hydrolysis of PI by *OsPI-PLC4* required Ca^{2+} , with the maximum activity being 50 mM Ca^{2+} [54] but *OsPI-PLC1* maximal activity was observed at 100 μM Ca^{2+} [57]. Distinct requirements for Ca^{2+} ions in tomato *SIPLC2*, *SIPLC4*, and *SIPLC5* enzymes [70], and PI-PLC sensitivity to calcium entry into cells during cold stress action [71] support the well-known role of calcium in the modulation of PI-PLC. Additionally, PI-PLC regulation at the protein level was shown in response to melatonin in oat seeds [72] and by substrate supply produced by phosphoinositide kinases [73]. Taken together, PI-

PLC regulation by post-translational modifications, bound proteins, protein level, and small molecules could modulate the level of DAG production for subsequent modification of PA signal strength and its velocity. This is important for tight regulation of PA interaction with its targets.

Advances in defining the functions, regulation, and localization of non-specific phospholipase C isoforms

Non-specific phospholipase C (NPC or PC-PLC) usually hydrolyzes membrane structural phospholipids (i.e., phosphatidylcholine), producing diacylglycerol and phosphocholine [74]. However, it also catalyzes other types of reactions. Non-specific phospholipase C3 from *Raphanus sativus* produces phytoceramide 1-phosphate from glycosylinositol phosphoceramide [75]. *Arabidopsis* NPC6 hydrolyzes not only phosphatidylcholine but also galactolipids [76]. Plant NPC is simply organized (Fig. 2). NPC4 from *Arabidopsis* is divided into a phosphoesterase domain (PD) and a C-terminal domain (CTD). The previously uncharacterized CTD is indispensable for the full activity of NPC4. Mechanistically, CTD contributes to NPC4 activity mainly via the CTD α 1-PD interaction, which ultimately stabilizes the catalytic pocket in PD [21]. Ten orchid PC-PLC protein sequences (*PePC-PLC1*, *PePC-PLC2A*, *DcPC-PLC1A*, *DcPC-PLC2*, *DcPC-PLC1B*, *DcPC-PLC5*, *AsPC-PLC1*, *AsPC-PLC2*, *AsPC-PLC3*, and *AsPC-PLC5*) were reported to have signal peptides. *In silico* prediction indicated the presence of a transmembrane region in three proteins (*PePC-PLC1*, *DcPC-PLC1A*, and *AsPC-PLC2*) [22]. Six maize *ZmNPCs* had only a phosphoesterase domain, which contains two highly conserved motifs, ENRSFDxxxG and TxPNR, and two invariable motifs, DExxGxxDHV and GxRVPxxxxxP [19]. The structures of cotton *GhNPCs* were composed of the beta sheet and several alpha helices [77]. Members of orchid PC-PLC (characterized by the presence of a phosphodiesterase domain only) were observed with six beta-sheets in their tertiary structure, but *DcPC-PLC1A* was predicted to have a large number of variations in their protein sequence at the alpha-helix region. In PC-PLC proteins, variations in the beta-sheets were observed to be greater in comparison to the alpha-helix, except in *PePC-PLC1* and *AsPC-PLC1* [22]. Therefore, specific structure determinants within PC-PLC could be involved in the regulation of the enzyme by interaction with modu-

lators or in modulating its specific localization in cells, thus affecting the number of DAG precursors of PA molecules produced and thereby affecting the level of PA signal.

Results of recent investigations in different plants indicate that NPCs are differentially localized within the cells. Although *ZmNPCs* were predicted to be multi-localized, *ZmNPC3* was experimentally confirmed to be located in the cytosol [19]. *Glehnia littoralis* *GINPC3* was predominantly localized at the plasma membrane, with some localization associated with the tonoplast [78]. In addition, rice NPCs were also localized at the cell periphery and plasma membrane of protoplasts [74]. Peach *PpNPC1* was experimentally located in the plasma membrane [79]. Also, subcellular localization predictive studies showed that most orchid NPC proteins were localized in the cytoplasm, nucleus, and mitochondria [22]. *AtNPC6* was found to be associated with chloroplast and microsomal fractions [76], but *AtNPC2* was present predominantly in Golgi apparatus, with a minor extent in other compartments of the secretory pathway, such as the endoplasmic reticulum or some post-Golgi compartments [80]. It can be proposed that specific PC-PLC localization in cells could determine the site of DAG production for subsequent PA generation and signaling and PAs vicinity to target proteins.

Some evidence has been reported regarding the post-translational regulation of NPC. The acylation of NPC4 was detected using NPC4 isolated from *Arabidopsis* and is important for membrane association and the hydrolysis of phosphosphingolipid glycosyl inositol phosphoryl ceramide during phosphate deficiency [81]. Changes in N-glycosylation were found for tomato NPC1 during ripening [35]. Taken together, PC-PLC regulation by post-translational modifications could potentially modulate the level of DAG production for subsequent modulation of PA signal strength and its velocity, which is important for tight regulation of PA interaction with its targets.

Advances in defining the functions, regulation, and localization of diacylglycerol kinases

DGK structure and localization. DGK carries out substrate diacylglycerol phosphorylation, producing phosphatidic acid as a product. DGKs in plants are represented by heterogenic enzyme families. These enzymes are characterized by a multidomain structure containing a range of functional mo-

tifs (Fig. 2). For example, DGK from common beans contains DAG/phorbol ester (PE)-binding domain 1, DAG/PE-binding domain 2, and the diacylglycerol kinase accessory (DGKa) domain [23]. *Ta*DGK harbored a diacylglycerol kinase catalytic domain (DGKc) and one accessory domain (DGKa) near the N-terminus, as well as an upstream basic region, an extCRD-like domain, and upstream basic regions near the C-terminus [82]. Almost all soybean DGKs contain an ATP-binding sequence (GXGXXG) in their catalytic domain (DGKc). C-terminal DAG/PE-binding domain C1 contains an additional 15-amino acid sequence (extCRD), whereas the sequence rich in basic amino acids is localized near the N-terminal C1 domain [26]. Rape DGKs also possess a conserved ATP-binding site, C1 domains, and an extCRD domain. However, *Bna*DGK2–1 lacks an N-terminal sequence rich in basic amino acids and an extended C1 sequence [24]. All DGKs in rice (*Os*DGK1–8) contain catalytic and accessory domains, but *Os*DGK4, *Os*DGK5, and *Os*DGK6 also contain two C1 domains. Among all known DGKs, only *Os*DGK6 contains a specific domain PPR instead of C1 domains, and this domain is known to play a role in macromolecular interactions [25]. Therefore, specific structure determinants within DGKs could be involved in the regulation of the enzyme by interaction with modulators or in modulating its specific localization in cells, thus affecting the number of PA molecules produced and the strength of the PA signal.

DGK localization in cells reflects the site of PA formation. Application of PA biosensors and pharmacological analysis suggest a role of DGK in the formation of 50–60% basal levels of PA localized in the plasma membrane and nucleus in the root epidermal cells of *Arabidopsis thaliana* [83]. Experimental evidence indicates that *Arabidopsis* DGK2 and DGK4 were localized to the endoplasmic reticulum and were involved in PA production for pollen tube growth [84]. Artificial expression of DGK2-GFP, DGK3-GFP, and DGK5-GFP in *Arabidopsis* indicated their localization in the cytosol [85]. In tobacco pollen tubes, DGK1–3 was observed to be localized within the endoplasmic reticulum, DGK4 – in the cytosol, DGK6 – in the cytoplasm, DGK5, DGK7, and DGK8 – on the plasma membrane. DGK5 was suggested to bind to the phospholipid bilayer by catalytic and accessory domains. Glycine-118 was proposed to be the key amino acid in DGK5 for binding to membranes, enzymatic activity, and

regulation of pollen tube growth [86]. Other types of data on DGK localization are based on *in silico* predictions. Wheat DGKs were predicted to localize to the chloroplast, cytoplasm, and nucleus. *Ta*DGK2A and *Ta*DGK5B were expressed in the nucleus and cytomembrane, while *Ta*DGK3A and *Ta*DGK5A2 were mostly expressed in the cytomembrane based on confocal microscopy [82]. Also, *Gm*DGK2, *Gm*DGK11, and *Gm*DGK12 in soybean are predicted to be localized in the plasma membrane and endoplasmic reticulum, whereas *Gm*DGK1, *Gm*DGK3–4, *Gm*DGK8–9, *Gm*DGK5–7, and *Gm*DGK10 are localized in the cytoplasm and nucleus; *Gm*DGK10 is mostly localized in the nucleus [26]. Among different *Brassica* species (*Brassica napus* and *Brassica oleracea*), *Bna*DGK1–2, *Bna*DGK2–1, *Bol*DGK1–2, *Bol*DGK2–2 can be localized in nucleus, DGK3 and DGK7 – in peroxisome, DGK4 – in chloroplast, *Bna*DGK3–3 and *Bol*DGK3–2 – in mitochondria, whereas DGK5 and DGK6 – in peroxisomes and cytosol, respectively [24]. Prediction of apple DGK localization by *in silico* analysis suggests that *Md*DGK1, *Md*DGK3, and *Md*DGK7 can be localized in the nucleus and membranes of the endoplasmic reticulum [87]. Therefore, specific DGK localization in cells could determine the site of starting PA signaling and its vicinity to target proteins.

Regulatory mechanisms of plant DGKs. One mechanism of DGK regulation in plants is the modification of their protein structure. Among them, phosphorylation is one of the known mechanisms of DGK regulation. Cadmium-, cold-, and MAPK11-induced protein phosphorylation was observed for some tomato DGKs [43, 60, 88]. Exogenous peptide systemin [89] and TOR kinase [90] are other regulators that induce changes in DGK phosphorylation in plants. Bolting induced by high temperatures induces changes in lettuce DGK (A0A2J6JMK6) phosphorylation [91]. DGK phosphorylation during fruit development was reported in pepper [62]. Phosphorylation changes in *Arabidopsis* DGK5 and DGK7 were observed in systemic leaves in response to the bacteria *Pseudomonas syringae* pv. *maculicola* ES4326 [92]. C-terminal proteolysis was found for DGK (Solyc10g008640) from tomato [66]. Another mechanism of DGK regulation is a modulation of protein stability that was found for pear DGK4 [93]. Analysis of protein-protein interaction indicated that, for example, *Arabidopsis* *At*DGK2 interacts physically with calmodulin CAM1 [94], whereas *At*DGK3 binds to MAP kinase MAPK6 [95], A-subunit of

splicing factor U2af, and cytoskeletal protein myosin [96]. Results of pharmacological analysis suggest that calcium is involved in the activation of PA accumulation in response to the elicitors cryptogein [97] and flagellin [98], but whether calcium directly affects DGK in these responses needs further investigation. Summing up, DGK regulation by post-translational modifications, bound proteins, and protein stability could modulate the level of PA signal strength and its velocity, which is important for tight regulation of PA interaction with its targets.

Role of PA and PA-producing enzymes in plant stress signaling

Specific enzymes of PA production and downstream PA-binding proteins are involved in, for example, hormone [99] and stress signaling in plants (Fig. 3, Table). In Table, we briefly summarize recent data on the genetically supported functions of the individual isoforms of PA-producing enzymes in stress signaling and PA production and the specific PA targets involved. These data suggest that the induction of cellular responses by different stressors may be coded by each specific subset of the isoforms of PA signaling enzymes and PA targets. Despite the successes and analysis of PI-PLC isoforms in plant responses to a number of stresses, the question of the involvement of individual PI-PLC isoforms is being intensively analyzed [100]. However, redundancy among different isoforms of PA-producing enzymes is also sometimes possible during stress signaling.

This was shown, for example, for PLD during effector-triggered immunity [101]. In addition, gene expression of PLD in response to elicitors and immunity inducers [102] and DGK in response to biotic and abiotic stressors [103] together with methylation of their genes [104] form the transcriptional control machinery of PA signaling specificity.

PA biosensor studies indicate that salt stress induces rapid PA accumulation with similar velocity but different degrees in plasma membranes of cells in different root zones [135]. Cold-induced PA also occurs at the plasma membrane [83], whereas heat stress induces rapid nuclear translocation of PA [4]. Fungal toxin botrydial [136] and elicitor chitosan [137] evoke rapid monophasic PA accumulation by PI-PLC/DGK and PLD pathways. Other elicitors induce PA accumulation mainly via the PI-PLC/DGK pathway [97]. Therefore, different dynamics of PA formation, in addition to specific sites of PA formation, is another level of PA signaling specificity.

Pathways downstream to PA-binding proteins may additionally specify PA signals into respective responses. For example, salicylic acid induces PLD-mediated translocation of the salicylic acid receptor, NPR1, to the nucleus [138]. PA accumulated in response to elicitors has been suggested to mediate elicitor-induced ROS and phytoalexin accumulation as well as elicitor-responsive defense gene expression [97]. Also, rapid elicitor-induced pH changes [70, 139] and endocytosis of the flagellin receptor [70] function downstream of PI-PLC. Flagellin-in-

Table. Plant PA signaling in response to stress. Shown here are PLD, PI-PLC, NPC, and DGK isoforms, as well as PA-binding proteins involved in stress signaling pathways. Negative regulators of stress tolerance are shown in italics

Stress type	Enzyme isoform involved	Known PA-binding protein involved	References
Salt stress	<i>AtPLD</i> α1/δ, <i>AtPLD</i> ζ1-2, <i>AtNPC</i> 4, <i>OsPI-PLC</i> 1/4, <i>OsDGK</i> s	<i>AtMAP</i> 65-1, <i>AtPIN</i> OID, <i>AtMKK</i> 7/9, <i>GMK</i> 1, <i>AtSOS</i> 2, <i>AtCHC</i> , <i>AtANTH</i> , <i>AtKAB</i> 1, ribosomal proteins (S3, L30), <i>AtGAPDH</i> , <i>AtPI4K</i> γ	[4, 25, 54, 57, 105-116]
Cold	<i>AtDGK</i> 2-3, <i>AtDGK</i> 5, <i>OsPLD</i> α1	<i>OsMPK</i> 6, <i>OsSIZ</i> 1, <i>AtRbohD</i>	[85, 117]
Heat stress	<i>AtPLD</i> α1/δ, <i>AtNPC</i> 1, <i>AtPLC</i> 5	<i>AtGAPDH</i>	[4, 118-121]
Wounding	<i>GhPLD</i> α/δ	<i>ZmCPK</i> 11	[122, 123]
Hypoxia	<i>AtPLD</i> α1/δ, <i>AtPLD</i> ζ1-2	<i>AtCPK</i> 12, <i>AtMPK</i> 3/ <i>AtMPK</i> 6	[40, 124-126]
Biotic stress	<i>NbPLD</i> α1, <i>AtPLD</i> α1, <i>AtPLD</i> β1-2, <i>AtPLD</i> γ1, <i>AtPLD</i> δ, <i>SIPLD</i> α1/γ, <i>NtDGK</i> 5, <i>AtDGK</i> 5, <i>AtPI-PLC</i> 2, <i>NbPLC</i> 3, <i>SIPLC</i> 2, <i>AtNPC</i> 2	<i>AtPDK</i> 1, <i>AtWIPK</i> , <i>AtCP</i>	[9, 30, 32, 36, 80, 97, 98, 127-134]

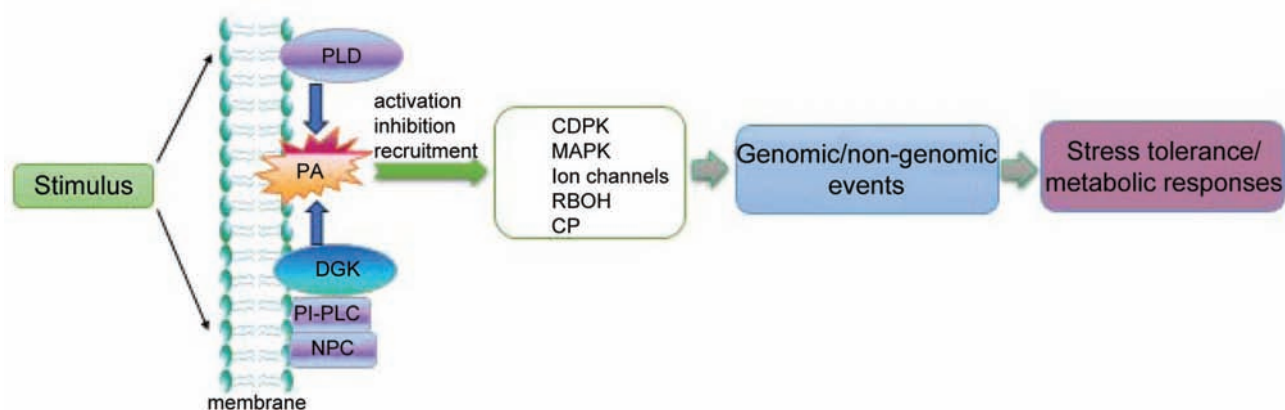


Fig. 3. A general model of plant PA signaling in plant cells. Specific extracellular stimuli induce the activation of a particular PA-producing enzyme isoform(s) by affecting localization, post-translational modification, protein-protein interaction, protein stability, or interaction with small regulatory molecules. Then, PA produced at a specific location binds to and modulates the localization and/or activity of target proteins. Subsequent genomic and/or non-genomic events coded by respective PA-target proteins further induce metabolic responses, leading to stress tolerance

duced changes in phosphorylation of some proteins are also found to be downstream of PLD in legume plants [140]. In addition, receptor-like kinase CRK2 [133], and auxin transporter AUX1 cellular relocation [116] are also regulated downstream of salt stress-regulated PLD and PA formation. Finally, according to the analysis of gene expression, the PI-PLC and PLD pathways are upstream of different cold-induced signaling pathways leading to cold responses [141].

Conclusion. It is obvious that the specificity of phosphatidic acid formation and signaling in plants is multifaceted and complex. Despite numerous studies using modern methods of analyzing the factors that can activate (or potentially activate) phosphatidic acid formation and signaling in plant cells, new methods and approaches are needed to form a more holistic picture of this process. It can be suggested that specific catalytic activity modulated by enzyme structure, presence/absence of specific structural determinants (domains, functional motifs) of PA-producing enzymes, post-translational modification, their time and spatially regulated gene expression, and localization should be more deeply investigated. PA produced in response to the stimuli participates in genomic and non-genomic signaling events regulating gene expression, ion transport, cytoskeleton dynamics, and metabolic enzyme activity. To perform this, PA, as a phospholipid second messenger, directly modulates specific target pro-

teins and enzymes, regulating their function, thus specifically directing and modulating downstream signaling events. Different plants possess their own isoform landscape of PA-producing enzymes and their intracellular localization, suggesting that signaling responses in cells to different factors action could be additionally regulated at the level of PA signaling enzymes.

The insufficient chromatographic resolution of anionic phospholipids, which include PA, significantly complicates the understanding of their role in the regulation of plant cell metabolism [142]. Important progress in understanding the localization and formation of phosphatidic acid in plant cells has certainly been achieved over the past year, thanks to new methodological approaches [100, 142]. These and other research findings will contribute to a more in-depth study of spatial and temporal changes in phosphatidic acid metabolism in plant cells during their growth, development, and formation of adaptive changes under the influence of numerous environmental stressors.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

Funding. The work was supported by grants of NAS Ukraine: № 2.1.10.32-20-24 and a grant for the preparation of Yaroslava Bukhonska's PhD thesis.

ФОРМУВАННЯ ФОСФАТИДНОЇ КИСЛОТИ ТА ПЕРЕДАЧА СИГНАЛІВ У КЛІТИНАХ РОСЛИН

Я. С. Колесников, С. В. Кретинін,
В. С. Кравець, Я. К. Бухонська✉

Інститут біоорганічної хімії та нафтохімії
ім. В. П. Кухаря НАН України, Київ;
✉e-mail: yasya.yaroslavka@gmail.com

В огляді представлено оновлену інформацію про структуру, локалізацію та регуляцію ензимів формування фосфатидних кислот (ФК) фосфоліпази D, фосфоінозитид-специфічної та неспецифічної фосфоліпази C та діацилгліцеролкінази. Обговорюється специфічна роль ФК та ензимів, що продукують ФК, у процесах трансдукції сигналів у рослинах під час стресу.

Ключові слова: фосфатидна кислота, клітини рослин, фосфоліпази D і C, діацилгліцеролкіназа, зв'язувальні протеїни, сигналювання.

References

1. 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(6): 3227.
2. Yao S, Kim SC, Li J, Tang S, Wang X. Phosphatidic acid signaling and function in nuclei. *Prog Lipid Res.* 2024; 93: 101267.
3. 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(3): 1434-1446.
4. Li T, Xiao X, Liu Q, Li W, Li L, Zhang W, Munnik T, Wang X, Zhang Q. Dynamic responses of PA to environmental stimuli imaged by a genetically encoded mobilizable fluorescent sensor. *Plant Commun.* 2023; 4(3): 100500.
5. Guan B, Jiang YT, Lin DL, Lin WH, Xue HW. Phosphatidic acid suppresses autophagy through competitive inhibition by binding GAPC (glyceraldehyde-3-phosphate dehydrogenase) and PGK (phosphoglycerate kinase) proteins. *Autophagy.* 2022; 18(11): 2656-2670.
6. Pandit S, Goel R, Mishra G. Phosphatidic acid binds to and stimulates the activity of ARGH2 from *Arabidopsis*. *Plant Physiol Biochem.* 2022; 185: 344-355.
7. Cao H, Gong R, Yuan S, Su Y, Lv W, Zhou Y, Zhang Q, Deng X, Tong P, Liang S, Wang X, Hong Y. Phospholipase Da6 and phosphatidic acid regulate gibberellin signaling in rice. *EMBO Rep.* 2021; 22(10): e51871.
8. Takai Y, Hasi RY, Matsumoto N, Fujita C, Ali H, Hayashi J, Kawakami R, Aihara M, Ishikawa T, Imai H, Wakida M, Ando K, Tanaka T. Degradation of glycosylinositol phosphoceramide during plant tissue homogenization. *J Biochem.* 2023; 175(1): 115-124.
9. Hu Z, Shi J, Feng S, Wu X, Shao S, Shi K. Plant N-acyl ethanolamines play a crucial role in defense and its variation in response to elevated CO₂ and temperature in tomato. *Hortic Res.* 2022; 10(1): uhac242.
10. Zhang H, Yu Y, Wang S, Yang J, Ai X, Zhang N, Zhao X, Liu X, Zhong C, Yu H. Genome-wide characterization of phospholipase D family genes in allotetraploid peanut and its diploid progenitors revealed their crucial roles in growth and abiotic stress responses. *Front Plant Sci.* 2023; 14: 1102200.
11. Fang S, Han X, Yuan P, Song C, Song S, Jiao J, Wang M, Zheng X, Bai T. Genome-wide analysis of the apple *PLD* gene family and a functional characterization of *MdPLD17* in drought tolerance. *Sci Horticult.* 2023; 321: 112311.
12. Wei J, Shao W, Liu X, He L, Zhao C, Yu G, Xu J. Genome-wide identification and expression analysis of phospholipase D gene in leaves of sorghum in response to abiotic stresses. *Physiol Mol Biol Plants.* 2022; 28(6): 1261-1276.
13. Sadat MA, Ullah MW, Hossain MS, Ahmed B, Bashir KK. Genome-wide in silico identification of phospholipase D (*PLD*) gene family from *Corchorus capsularis* and *Corchorus olitorius*: reveals their responses to plant stress. *J Genet Eng Biotechnol.* 2022; 20(1): 28.
14. Yuan Y, Yu J, Kong L, Zhang W, Hou X, Cui G. Genome-wide investigation of the *PLD* gene family in alfalfa (*Medicago sativa* L.): identification, analysis and expression. *BMC Genomics.* 2022; 23(1): 243.
15. Laureano G, Santos C, Gouveia C, Matos AR, Figueiredo A. Grapevine-associated lipid signalling is specifically activated in an *Rpv3*

- background in response to an aggressive *P. viticola* Pathovar. *Cells*. 2023; 12(3): 394.
16. Hong K, Zhang L, Zhan R, Huang B, Song K, Jia Z. Identification and characterization of phospholipase D genes putatively involved in internal browning of pineapple during postharvest storage. *Front Plant Sci*. 2017; 8: 913.
 17. 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(1): 212-226.
 18. Liu P, Gu J, Cui X, Fu H, Wang F, Qi M, Sun Z, Li T, Liu Y. Genome-wide investigation of the phospholipase C gene family in *Solanum lycopersicum* and abiotic stress analysis. *Environ Exp Bot*. 2023; 210: 105336.
 19. Zhu J, Zhou Y, Li J, Li H. Genome-wide investigation of the phospholipase C gene family in *Zea mays*. *Front Genet*. 2021; 11: 611414.
 20. Zhu L, Dou L, Shang H, Li H, Yu J, Xiao G. *GhPIPLC2D* promotes cotton fiber elongation by enhancing ethylene biosynthesis. *iScience*. 2021; 24(3): 102199.
 21. Fan R, Zhao F, Gong Z, Chen Y, Yang B, Zhou C, Zhang J, Du Z, Wang X, Yin P, Guo L, Liu Z. Insights into the mechanism of phospholipid hydrolysis by plant non-specific phospholipase C. *Nat Commun*. 2023; 14(1): 194.
 22. Kanchan M, Ramkumar TR, Himani, Sembi JK. Genome-wide characterization and expression profiling of the *Phospholipase C (PLC)* gene family in three orchids of economic importance. *J Genet Eng Biotechnol*. 2021; 19(1): 124.
 23. Yeken MZ, Özer G, Çiftçi V. Genome-wide identification and expression analysis of DGK (Diacylglycerol Kinase) genes in common bean. *J Plant Growth Regul*. 2023; 42: 2558-2569.
 24. Tang F, Xiao Z, Sun F, Shen S, Chen S, Chen R, Zhu M, Zhang Q, Du H, Lu K, Li J, Qu C. Genome-wide identification and comparative analysis of diacylglycerol kinase (DGK) gene family and their expression profiling in *Brassica napus* under abiotic stress. *BMC Plant Biol*. 2020; 20(1): 473.
 25. Ge H, Chen C, Jing W, Zhang Q, Wang H, Wang R, Zhang W. The rice diacylglycerol kinase family: functional analysis using transient RNA interference. *Front Plant Sci*. 2012; 3: 60.
 26. Carther KF, Ketehouli T, Ye N, Yang YH, Wang N, Dong YY, Yao N, Liu XM, Liu WC, Li XW, Wang FW, Li HY. Comprehensive genomic analysis and expression profiling of diacylglycerol kinase (DGK) gene family in soybean (*Glycine max*) under abiotic stresses. *Int J Mol Sci*. 2019; 20(6): 1361.
 27. 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(1): 142-158.
 28. Ma C, Zhang Q, Lv J, Qiao K, Fan S, Ma Q, Zhang C. Genome-wide analysis of the phospholipase D family in five cotton species, and potential role of *GhPLD2* in fiber development and anther dehiscence. *Front Plant Sci*. 2021; 12: 728025.
 29. Cao H, Liu Q, Liu X, Ma Z, Zhang J, Li X, Shen L, Yuan J, Zhang Q. Phosphatidic acid regulates ammonium uptake by interacting with AMMONIUM TRANSPORTER 1;1 in *Arabidopsis*. *Plant Physiol*. 2023; 193(3): 1954-1969.
 30. Xing J, Li X, Wang X, Lv X, Wang L, Zhang L, Zhu Y, Shen Q, Baluška F, Šamaj J, Lin J. Secretion of phospholipase D δ functions as a regulatory mechanism in plant innate immunity. *Plant Cell*. 2019; 31(12): 3015-3032.
 31. Shimamura R, Ohashi Y, Taniguchi YY, Kato M, Tsuge T, Aoyama T. *Arabidopsis* PLD ζ 1 and PLD ζ 2 localize to post-Golgi membrane compartments in a partially overlapping manner. *Plant Mol Biol*. 2022; 108(1-2): 31-49.
 32. Schlöffel MA, Salzer A, Wan W., van Wijk R, Šemanjski M, Symeonidi E, Slaby P, Kilian J, Maček B, Munnik T, Gust AA. The BIR2/BIR3-interacting phospholipase D gamma 1 negatively regulates plant immunity. *Plant Physiol*. 2019; 183(1): 371-384.
 33. Ying S, Scheible WR, Lundquist PK. A stress-inducible protein regulates drought tolerance and flowering time in *Brachypodium* and *Arabidopsis*. *Plant Physiol*. 2023; 191(1): 643-659.
 34. Zhang K, Shi W, Zheng X, Liu X, Wang L, Riemann M, Heintz D, Nick P. A rice tubulin tyrosine ligase like 12 regulates phospholipase D activity and tubulin synthesis. *Plant Sci*. 2022; 316: 111155.
 35. Zhang X, Tang H, Du H, Liu Z, Bao Z, Shi Q. Comparative N-glycoproteome analysis provides novel insights into the regulation mechanism in

- tomato (*solanum lycopersicum* L.) During fruit ripening process. *Plant Sci.* 2020; 293: 110413.
36. Lin J, Zhao J, Du L, Wang P, Sun B, Zhang C, Shi Y, Li H, Sun H. Activation of MAPK-mediated immunity by phosphatidic acid in response to positive-strand RNA viruses. *Plant Commun.* 2024; 5(1): 100659.
 37. 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(10): 1005-1016.
 38. Yang J, Zheng Q, Wang Y, Wu T, Li W, Qiu C, Xu X, Zhang X, Han Z, Zhang X. GSH-dependent PTMs of proteins differ significantly between ontogenetic phases of apple trees. *J Plant Growth Regul.* 2023; 42: 3405-3418.
 39. Nounurai P, Afifah A, Kittisenachai S, Roytrakul S. Phosphorylation of CAD1, PLDdelta, NDT1, RPM1 Proteins Induce Resistance in Tomatoes Infected by *Ralstonia solanacearum*. *Plants (Basel).* 2022; 11(6): 726.
 40. Zhou Y, Zhou DM, Yu WW, Shi LL, Zhang Y, Lai YX, Huang LP, Qi H, Chen QF, Yao N, Li JF, Xie LJ, Xiao S. Phosphatidic acid modulates MPK3- and MPK6-mediated hypoxia signaling in *Arabidopsis*. *Plant Cell.* 2022; 34(2): 889-909.
 41. Amagai A, Honda Y, Ishikawa S, Hara Y, Kuwamura M, Shinozawa A, Sugiyama N, Ishihama Y, Takezawa D, Sakata Y, Shinozaki K, Umezawa T. Phosphoproteomic profiling reveals ABA-responsive phosphosignaling pathways in *Physcomitrella patens*. *Plant J.* 2018; 94(4): 699-708.
 42. Shinozawa A, Otake R, Takezawa D, Umezawa T, Komatsu K, Tanaka K, Amagai A, Ishikawa S, Hara Y, Kamisugi Y, Cuming AC, Hori K, Ohta H, Takahashi F, Shinozaki K, Hayashi T, Taji T, Sakata Y. SnRK2 protein kinases represent an ancient system in plants for adaptation to a terrestrial environment. *Commun Biol.* 2019; 2: 30.
 43. Hsu CC, Zhu Y, Arrington JV, Paez JS, Wang P, Zhu P, Chen IH, Zhu JK, Tao WA. Universal plant phosphoproteomics workflow and its application to tomato signaling in response to cold stress. *Mol Cell Proteomics.* 2018; 17(10): 2068-2080.
 44. Wang C, Guo H, He X, Zhang S, Wang J, Wang L, Guo D, Guo X. Scaffold protein GhMORG1 enhances the resistance of cotton to *Fusarium oxysporum* by facilitating the MKK6-MPK4 cascade. *Plant Biotechnol J.* 2020; 18(6): 1421-1433.
 45. Heidari P, Puresmaeli F, Vafaei Y, Ahmadizadeh M, Ensani M, Ahmadiania H. Comparative analysis of phospholipase D (PLD) gene family in *Camelina sativa* and *Brassica napus* and its responses in camelina seedlings under salt stress. *Agronomy.* 2023; 13(10): 2616.
 46. Chen X, Xu Q, Duan Y, Liu H, Chen X, Huang J, Luo C, Zhou DX, Zheng L. Ustilaginoidea virens modulates lysine 2-hydroxyisobutyrylation in rice flowers during infection. *J Integr Plant Biol.* 2021; 63(10): 1801-1814.
 47. Pan C, Li X, Yao S, Luo S, Liu S, Wang A, Xiao D, Zhan J, He L. S-nitrosated proteomic analysis reveals the regulatory roles of protein S-nitrosation and S-nitrosoglutathione reductase during Al-induced PCD in peanut root tips. *Plant Sci.* 2021; 308: 110931.
 48. Liao X, Li Y, Hu Z, Lin Y, Zheng B, Ding J. Poplar acetylome profiling reveals lysine acetylation dynamics in seasonal bud dormancy release. *Plant Cell Environ.* 2021; 44(6): 1830-1845.
 49. Song P, Jia Q, Chen L, Jin X, Xiao X, Li L, Chen H, Qu Y, Su Y, Zhang W, Zhang Q. Involvement of *Arabidopsis* phospholipase D δ in regulation of ROS-mediated microtubule organization and stomatal movement upon heat shock. *J Exp Bot.* 2020; 71(20): 6555-6570.
 50. Wilmowicz E, Kućko A, Pokora W, Kapusta M, Jasieniecka-Gazarkiewicz K, Tranbarger TJ, Wolska M, Panek K. EPIP-evoked modifications of redox, lipid, and pectin homeostasis in the abscission zone of lupine flowers. *Int J Mol Sci.* 2021; 22(6): 3001.
 51. Ribeiro DG, Bezerra AC, Santos IR, Grynberg P, Fontes W, de Souza Castro M, de Sousa MV, Lisei-de-Sá ME, Grossi-de-Sá MF, Franco OL, Mehta A. Proteomic insights of cowpea response to combined biotic and abiotic stresses. *Plants (Basel).* 2023; 12(9): 1900.
 52. Prasad K, Yogendra K, Sanivarapu H, Rajasekaran K, Cary JW, Sharma KK, Bhatnagar-Mathur P. Multiplexed host-induced gene silencing of *Aspergillus flavus* genes confers aflatoxin resistance in groundnut. *Toxins (Basel).* 2023; 15(5): 319.
 53. Oblozinsky M, Bezakova L, Mansfeld J, Heilmann I, Ulbrich-Hofmann R. Differences in the effect of phosphatidylinositol

- 4,5-bisphosphate on the hydrolytic and transphosphatidylolation activities of membrane-bound phospholipase D from poppy seedlings. *Plant Physiol Biochem.* 2013; 69: 39-42.
54. Deng X, Yuan S, Cao H, Lam SM, Shui G, Hong Y, Wang X. Phosphatidylinositol-hydrolyzing phospholipase C4 modulates rice response to salt and drought. *Plant Cell Environ.* 2019; 42(2): 536-548.
 55. Yu M, Cao C, Yin X, Liu X, Yang D, Gong C, Wang H, Wu Y. The rice phosphoinositide-specific phospholipase C3 is involved in responses to osmotic stresses via modulating ROS homeostasis. *Plant Sci.* 2021; 313: 111087.
 56. Sagar S, Biswas DK, Singh A. Genomic and expression analysis indicate the involvement of phospholipase C family in abiotic stress signaling in chickpea (*Cicer arietinum*). *Gene.* 2020; 753: 144797.
 57. Li L, Wang F, Yan P, Jing W, Zhang C, Kudla J, Zhang W. A phosphoinositide-specific phospholipase C pathway elicits stress-induced Ca^{2+} signals and confers salt tolerance to rice. *New Phytol.* 2017; 214(3): 1172-1187.
 58. Wu Q, Fan Z, Qi F, Li D, Zhang Z, Chen Y, Huang Y, Lin Y, Lai Z. Genome-wide identification, evolution analysis of PI-PLC family and their expression patterns in response to different hormones and growth in banana (*Musa L.*). *Trop Plant Biol.* 2023; 16: 187-198.
 59. Kong J, Chen R, Liu R, Wang W, Wang S, Zhang J, Yang N. PLC1 mediated Cycloastragenol-induced stomatal movement by regulating the production of NO in *Arabidopsis thaliana*. *BMC Plant Biol.* 2023; 23(1): 571.
 60. Marques DN, Stolze SC, Harzen A, Nogueira ML, Batagin-Piotto KD, Piotto FA, Mason C, Azevedo RA, Nakagami H. Comparative phosphoproteomic analysis of tomato genotypes with contrasting cadmium tolerance. *Plant Cell Rep.* 2021; 40(10): 2001-2008.
 61. Lu ZS, Chen QS, Zheng QX, Shen JJ, Luo ZP, Fan K, Xu SH, Shen Q, Liu PP. Proteomic and phosphoproteomic analysis in tobacco mosaic virus-infected tobacco (*Nicotiana tabacum*). *Biomolecules.* 2019; 9(2): 39.
 62. Liu Z, Lv J, Liu Y, Wang J, Zhang Z, Chen W, Song J, Yang B, Tan F, Zou X, Ou L. Comprehensive phosphoproteomic analysis of pepper fruit development provides insight into plant signaling transduction. *Int J Mol Sci.* 2020; 21(6): 1962.
 63. Han R, Wei Y, Xie Y, Liu L, Jiang C, Yu Y. Quantitative phosphoproteomic analysis provides insights into the aluminum-responsiveness of Tamba black soybean. *PLoS One.* 2020; 15(8): e0237845.
 64. Smith S, Zhu S, Joos L, Roberts I, Nikonorova N, Vu LD, Stes E, Cho H, Larrieu A, Xuan W, Goodall B, van de Cotte B, Waite JM, Rigal A, Ramans Harborough S, Persiau G, Vanneste S, Kirschner GK, Vandermarliere E, Martens L, Stahl Y, Audenaert D, Friml J, Felix G, Simon R, Bennett MJ, Bishopp A, De Jaeger G, Ljung K, Kepinski S, Robert S, Nemhauser J, Hwang I, Gevaert K, Beeckman T, De Smet I. The CEP5 Peptide Promotes Abiotic Stress Tolerance, As Revealed by Quantitative Proteomics, and Attenuates the AUX/IAA Equilibrium in *Arabidopsis*. *Mol Cell Proteomics.* 2020; 19(8): 1248-1262.
 65. Sun J, Qiu C, Qian W, Wang Y, Sun L, Li Y, Ding Z. Ammonium triggered the response mechanism of lysine crotonylome in tea plants. *BMC Genomics.* 2019; 20(1): 340.
 66. Li Q, Zhang Y, Huang J, Wu Z, Tang L, Huang L, Zhang X. Basic strong cation exchange chromatography, BaSCX, a highly efficient approach for C-terminomic studies using lysargiNase digestion. *Anal Chem.* 2020; 92(7): 4742-4748.
 67. Wang N, Shi Y, Jiang Q, Li H, Fan W, Feng Y, Li L, Liu B, Lin F, Jing W, Zhang W, Shen L. A 14-3-3 protein positively regulates rice salt tolerance by stabilizing phospholipase C1. *Plant Cell Environ.* 2023; 46(4): 1232-1248.
 68. Bovin AD, Pavlova OA, Dolgikh AV, Leppyanen IV, Dolgikh EA. The role of heterotrimeric G-protein beta subunits during nodulation in *Medicago truncatula* Gaertn and *Pisum sativum* L. *Front Plant Sci.* 2022; 12: 808573.
 69. She J, Yan H, Yang J, Xu W, Su Z. croFGD: *Catharanthus roseus* functional genomics database. *Front Genet.* 2019; 10: 238.
 70. Abd-El-Haliem AM, Vossen JH, van Zeijl A, Dezhsetan S, Testerink C, Seidl MF, Beck M, Strutt J, Robatzek S, Joosten MH. Biochemical characterization of the tomato phosphatidylinositol-specific phospholipase C (PI-PLC) family and its role in plant immunity. *Biochim Biophys Acta.* 2016; 1861(9 Pt B): 1365-1378.

71. Ruelland E, Cantrel C, Gawer M, Kader JC, Zachowski A. Activation of phospholipases C and D is an early response to a cold exposure in *Arabidopsis* suspension cells. *Plant Physiol.* 2002; 130(2): 999-1007.
72. Yan H, Mao P. Comparative time-course physiological responses and proteomic analysis of melatonin priming on promoting germination in aged oat (*Avena sativa* L.) seeds. *Int J Mol Sci.* 2021; 22(2): 811.
73. Delage E, Ruelland E, Guillas I, Zachowski A, Puyaubert J. *Arabidopsis* type-III phosphatidylinositol 4-kinases $\beta 1$ and $\beta 2$ are upstream of the phospholipase C pathway triggered by cold exposure. *Plant Cell Physiol.* 2012; 53(3): 565-576.
74. 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(6): 985-1000.
75. Hasi RY, Ishikawa T, Sunagawa K, Takai Y, Ali H, Hayashi J, Kawakami R, Yuasa K, Aihara M, Kanemaru K, Imai H, Tanaka T. Nonspecific phospholipase C3 of radish has phospholipase D activity towards glycosylinositol phosphoceramide. *FEBS Lett.* 2022; 596(23): 3024-3036.
76. Cai G, Fan C, Liu S, Yang Q, Liu D, Wu J, Li J, Zhou Y, Guo L, Wang X. Nonspecific phospholipase C6 increases seed oil production in oilseed Brassicaceae plants. *New Phytol.* 2020; 226(4): 1055-1073.
77. Song J, Zhou Y, Zhang J, Zhang K. Structural, expression and evolutionary analysis of the non-specific phospholipase C gene family in *Gossypium hirsutum*. *BMC Genomics.* 2017; 18(1): 979.
78. Li L, Li N, Qi X, Bai Y, Chen Q, Fang H, Yu X, Liu D, Liang C, Zhou Y. Characterization of the *Glehnia littoralis* non-specific phospholipase C gene *GINPC3* and its involvement in the salt stress response. *Front Plant Sci.* 2021; 12: 769599.
79. Wang K, Li YL, Chen S. Genome-wide identification of phospholipase C related to chilling injury in peach fruit. *J Plant Biochem Biotechnol.* 2021; 30: 452-461.
80. Krčková Z, Kocourková D, Daněk M, Brouzdová J, Pejchar P, Janda M, Pokotylo I, Ott PG, Valentová O, Martinec J. The *Arabidopsis thaliana* non-specific phospholipase C2 is involved in the response to *Pseudomonas syringae* attack. *Ann Bot.* 2018; 121(2): 297-310.
81. Yang B, Zhang K, Jin X, Yan J, Lu S, Shen Q, Guo L, Hong Y, Wang X, Guo L. Acylation of non-specific phospholipase C4 determines its function in plant response to phosphate deficiency. *Plant J.* 2021; 106(6): 1647-1659.
82. Jia X, Si X, Jia Y, Zhang H, Tian S, Li W, Zhang K, Pan Y. Genomic profiling and expression analysis of the diacylglycerol kinase gene family in heterologous hexaploid wheat. *PeerJ.* 2021; 9: e12480.
83. Platre MP, Noack LC, Doumane M, Bayle V, Simon MLA, Maneta-Peyret L, Fouillen L, Stanislas T, Armengot L, Pejchar P, Caillaud MC, Potocký M, Čopić A, Moreau P, Jaillais Y. A combinatorial lipid code shapes the electrostatic landscape of plant endomembranes. *Dev Cell.* 2018; 45(4): 465-480.e11.
84. Angkawijaya AE, Nguyen VC, Gunawan F, Nakamura Y. A Pair of *Arabidopsis* diacylglycerol kinases essential for gametogenesis and endoplasmic reticulum phospholipid metabolism in leaves and flowers. *Plant Cell.* 2020; 32(8): 2602-2620.
85. Tan WJ, Yang YC, Zhou Y, Huang LP, Xu L, Chen QF, Yu LJ, Xiao S. DIACYLGLYCEROL ACYLTRANSFERASE and DIACYLGLYCEROL KINASE modulate triacylglycerol and phosphatidic acid production in the plant response to freezing stress. *Plant Physiol.* 2018; 177(3): 1303-1318.
86. Scholz P, Pejchar P, Fernkorn M, Škrabáľková E, Pleskot R, Biersch K, Munnik T, Potocký M, Ischebeck T. DIACYLGLYCEROL KINASE 5 regulates polar tip growth of tobacco pollen tubes. *New Phytol.* 2022; 233(5): 2185-2202.
87. Li Y, Tan Y, Shao Y, Li M, Ma F. Comprehensive genomic analysis and expression profiling of diacylglycerol kinase gene family in *Malus prunifolia* (Willd.) Borkh. *Gene.* 2015; 561(2): 225-234.
88. Song J, Shang L, Wang X, Xing Y, Xu W, Zhang Y, Wang T, Li H, Zhang J, Ye Z. MAPK11 regulates seed germination and ABA signaling in tomato by phosphorylating SnRKs. *J Exp Bot.* 2021; 72(5): 1677-1690.
89. Haj Ahmad F, Wu XN, Stintzi A, Schaller A, Schulze WX. The systemin signaling cascade as derived from time course analyses of the systemin-responsive phosphoproteome. *Mol Cell Proteomics.* 2019; 18(8): 1526-1542.

90. Chen Q, Qu M, Chen Q, Meng X, Fan H. Phosphoproteomics analysis of the effect of target of rapamycin kinase inhibition on *Cucumis sativus* in response to *Podosphaera xanthii*. *Plant Physiol Biochem.* 2023; 197: 107641.
91. Qin X, Li P, Lu S, Sun Y, Meng L, Hao J, Fan S. Phosphoproteomic analysis of lettuce (*Lactuca sativa* L.) reveals starch and sucrose metabolism functions during bolting induced by high temperature. *PLoS One.* 2020; 15(12): e0244198.
92. Zhou Q, Meng Q, Tan X, Ding W, Ma K, Xu Z, Huang X, Gao H. Protein phosphorylation changes during systemic acquired resistance in *Arabidopsis thaliana*. *Front Plant Sci.* 2021; 12: 748287.
93. Kong XX, Mei JW, Zhang J, Liu X, Wu JY, Wang CL. 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(12): 2123-2135.
94. Popescu SC, Popescu GV, Bachan S, Zhang Z, Seay M, Gerstein M, Snyder M, Dinesh-Kumar SP. Differential binding of calmodulin-related proteins to their targets revealed through high-density Arabidopsis protein microarrays. *Proc Natl Acad Sci USA.* 2007; 104(11): 4730-4735.
95. Altmann M, Altmann S, Rodriguez PA, Weller B, Elorduy Vergara L, Palme J, Marín-de la Rosa N, Sauer M, Wenig M, Villaécija-Aguilar JA, Sales J, Lin CW, Pandiarajan R, Young V, Strobel A, Gross L, Carbonnel S, Kugler KG, Garcia-Molina A, Bassel GW, Falter C, Mayer KFX, Gutjahr C, Vlot AC, Grill E, Falter-Braun P. Extensive signal integration by the phytohormone protein network. *Nature.* 2020; 583(7815): 271-276.
96. Arabidopsis Interactome Mapping Consortium. Evidence for network evolution in an Arabidopsis interactome map. *Science.* 2011; 333(6042): 601-607.
97. Cacas JL, Gerbeau-Pissot P, Fromentin J, Cantrel C, Thomas D, Jeannette E, Kalachova T, Mongrand S, Simon-Plas F, Ruelland E. Diacylglycerol kinases activate tobacco NADPH oxidase-dependent oxidative burst in response to cryptogin. *Plant Cell Environ.* 2017; 40(4): 585-598.
98. Kalachova T, Škrabálková E, Pateyron S, Soubigou-Taconnat L, Djafi N, Collin S, Sekereš J, Burketová L, Potocký M, Pejchar P, Ruelland E. DIACYLGLYCEROL KINASE 5 participates in flagellin-induced signaling in *Arabidopsis*. *Plant Physiol.* 2022; 190(3): 1978-1996.
99. Janda M, Planchais S, Djafi N, Martinec J, Burketova L, Valentova O, Zachowski A, Ruelland E. Phosphoglycerolipids are master players in plant hormone signal transduction. *Plant Cell Rep.* 2013; 32(6): 839-851.
100. van Hooren M, Darwish E, Munnik T. Stress- and phospholipid signalling responses in *Arabidopsis* PLC4-KO and -overexpression lines under salt- and osmotic stress. *Phytochemistry.* 2023; 216: 113862.
101. Johansson ON, Fahlberg P, Karimi E, Nilsson AK, Ellerström M, Andersson MX. Redundancy among phospholipase D isoforms in resistance triggered by recognition of the *Pseudomonas syringae* effector AvrRpm1 in *Arabidopsis thaliana*. *Front Plant Sci.* 2014; 5: 639.
102. Janda M, Ježková L, Nováková M, Valentová O, Burketová L, Šašek V. Identification of phospholipase D genes in *Brassica napus* and their transcription after phytohormone treatment and pathogen infection. *Biol Plant.* 2015; 59: 581-590.
103. Wang H, Yan Z, Yang M, Gu L. Genome-wide identification and characterization of the diacylglycerol kinase (DGK) gene family in *Populus trichocarpa*. *Physiol Mol Plant Pathol.* 2023; 127: 102121.
104. Li J, Wang J, Pang Q, Yan X. Analysis of N6-methyladenosine reveals a new important mechanism regulating the salt tolerance of sugar beet (*Beta vulgaris*). *Plant Sci.* 2023; 335: 111794.
105. Ben Othman A, Ellouzi H, Planchais S, De Vos D, Faiyue B, Carol P, Abdelly C, Savouré A. Phospholipases D ζ 1 and D ζ 2 have distinct roles in growth and antioxidant systems in *Arabidopsis thaliana* responding to salt stress. *Planta.* 2017; 246(4): 721-735.
106. Galvan-Ampudia CS, Julkowska MM, Darwish E, Gandullo J, Korver RA, Brunoud G, Haring MA, Munnik T, Vernoux T, Testerink C. Halotropism is a response of plant

- roots to avoid a saline environment. *Curr Biol.* 2013; 23(20): 2044-2050.
107. Kocourková D, Krčková Z, Pejchar P, Veselková Š, Valentová O, Wimalasekera R, Scherer GFE, Martinec J. The phosphatidylcholine-hydrolysing phospholipase C NPC4 plays a role in response of *Arabidopsis* roots to salt stress. *J Exp Bot.* 2011; 62(11): 3753-3763.
108. Zhang Q, Lin F, Mao T, Nie J, Yan M, Yuan M, Zhang W. Phosphatidic acid regulates microtubule organization by interacting with MAP65-1 in response to salt stress in *Arabidopsis*. *Plant Cell.* 2012; 24(11): 4555-4576.
109. Yu L, Nie J, Cao C, Jin Y, Yan M, Wang F, Liu J, Xiao Y, Liang Y, Zhang W. Phosphatidic acid mediates salt stress response by regulation of MPK6 in *Arabidopsis thaliana*. *New Phytol.* 2010; 188(3): 762-773.
110. 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(1): 250-271.
111. 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.
112. Im JH, Lee H, Kim J, Kim HB, Seyoung K, Kim BM, An CS. A salt stress-activated mitogen-activated protein kinase in soybean is regulated by phosphatidic acid in early stages of the stress response. *J Plant Biol.* 2012; 55: 303-309.
113. Im JH, Lee H, Kim J, Kim HB, An CS. Soybean MAPK, GMK1 is dually regulated by phosphatidic acid and hydrogen peroxide and translocated to nucleus during salt stress. *Mol Cells.* 2012; 34(3): 271-278.
114. Li J, Shen L, Han X, He G, Fan W, Li Y, Yang S, Zhang Z, Yang Y, Jin W, Wang Y, Zhang W, Guo Y. Phosphatidic acid-regulated SOS2 controls sodium and potassium homeostasis in *Arabidopsis* under salt stress. *EMBO J.* 2023; 42(8): e112401.
115. McLoughlin F, Arisz Steven A, Dekker Henk L, Kramer G, de Koster Chris G, Haring Michel 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(3): 573-581.
116. Korver RA, van den Berg T, Meyer AJ, Galvan-Ampudia CS, ten Tusscher KHWJ, Testerink C. Halotropism requires phospholipase D ζ 1-mediated modulation of cellular polarity of auxin transport carriers. *Plant Cell Environ.* 2020; 43(1): 143-158.
117. Huo C, Zhang B, Wang H, Wang F, Liu M, Gao Y, Zhang W, Deng Z, Sun D, Tang W. Comparative study of early cold-regulated proteins by two-dimensional difference gel electrophoresis reveals a key role for phospholipase D α 1 in mediating cold acclimation signaling pathway in rice. *Mol Cell Proteomics.* 2016; 15(4): 1397-1411.
118. Kim SC, Yao S, Zhang Q, Wang X. Phospholipase D δ and phosphatidic acid mediate heat-induced nuclear localization of glyceraldehyde-3-phosphate dehydrogenase in *Arabidopsis*. *Plant J.* 2022; 112(3): 786-799.
119. Annum N, Ahmed M, Imtiaz K, Mansoor S, Tester M, Saeed NA. $^{32}\text{P}_i$ labeled transgenic wheat shows the accumulation of phosphatidylinositol 4,5-bisphosphate and phosphatidic acid under heat and osmotic stress. *Front Plant Sci.* 2022; 13: 881188.
120. Mishkind M, Vermeer JEM, Darwish E, Munnik T. Heat stress activates phospholipase D and triggers PIP accumulation at the plasma membrane and nucleus. *Plant J.* 2009; 60(1): 10-21.
121. Krčková Z, Brouzdová J, Daněk M, Kocourková D, Rainteau D, Ruelland E, Valentová O, Pejchar P, Martinec J. *Arabidopsis* non-specific phospholipase C1: characterization and its involvement in response to heat stress. *Front Plant Sci.* 2015; 6: 928.
122. Klimecka M, Szczegielniak J, Godecka L, Lewandowska-Gnatowska E, Dobrowolska G, Muszyńska G. Regulation of wound-responsive calcium-dependent protein kinase from maize (ZmCPK11) by phosphatidic acid. *Acta Biochim Pol.* 2011; 58(4): 589-595.
123. Bourtsala A, Farmaki T, Galanopoulou D. Phospholipases D α and δ are involved in local and systemic wound responses of cotton (*G. hirsutum*). *Biochem Biophys Rep.* 2016; 9: 133-139.

124. Premkumar A, Lindberg S, Lager I, Rasmussen U, Schulz A. *Arabidopsis* PLDs with C2-domain function distinctively in hypoxia. *Physiol Plant*. 2019; 167(1): 90-110.
125. Lindberg S, Premkumar A, Rasmussen U, Schulz A, Lager I. Phospholipases AtPLD ζ 1 and AtPLD ζ 2 function differently in hypoxia. *Physiol Plant*. 2018; 162(1): 98-108.
126. Fan B, Liao K, Wang LN, Shi LL, Zhang Y, Xu LJ, Zhou Y, Li JF, Chen YQ, Chen QF, Xiao S. Calcium-dependent activation of CPK12 facilitates its cytoplasm-to-nucleus translocation to potentiate plant hypoxia sensing by phosphorylating ERF-VII transcription factors. *Mol Plant*. 2023; 16(6): 979-998.
127. Anthony RG, Khan S, Costa J, Pais MS, Bögre L. The *Arabidopsis* protein kinase PTI1-2 is activated by convergent phosphatidic acid and oxidative stress signaling pathways downstream of PDK1 and OXI1. *J Biol Chem*. 2006; 281(49): 37536-37546.
128. Li J, Henty-Ridilla JL, Staiger BH, Day B, Staiger CJ. Capping protein integrates multiple MAMP signalling pathways to modulate actin dynamics during plant innate immunity. *Nat Commun*. 2015; 6: 7206.
129. Pinosa F, Buhot N, Kwaaitaal M, Fahlberg P, Thordal-Christensen H, Ellerström M, Andersson MX. *Arabidopsis* phospholipase d δ is involved in basal defense and nonhost resistance to powdery mildew fungi. *Plant Physiol*. 2013; 163(2): 896-906.
130. D'Ambrosio JM, Couto D, Fabro G, Scuffi D, Lamattina L, Munnik T, Andersson MX, Álvarez ME, Zipfel C, Laxalt AM. Phospholipase C2 Affects MAMP-Triggered Immunity by Modulating ROS Production. *Plant Physiol*. 2017; 175(2): 970-981.
131. Perk EA, Arruebarrena Di Palma A, Colman S, Mariani O, Cerrudo I, D'Ambrosio JM, Robuschi L, Pombo MA, Rosli HG, Villareal F, Laxalt AM. CRISPR/Cas9-mediated phospholipase C 2 knock-out tomato plants are more resistant to *Botrytis cinerea*. *Planta*. 2023; 257(6): 117.
132. Takasato S, Bando T, Ohnishi K, Tsuzuki M, Hikichi Y, Kiba A. Phosphatidylinositol-phospholipase C3 negatively regulates the hypersensitive response via complex signaling with MAP kinase, phytohormones, and reactive oxygen species in *Nicotiana benthamiana*. *J Exp Bot*. 2023; 74(15): 4721-4735.
133. Hunter K, Kimura S, Rokka A, Tran HC, Toyota M, Kukkonen JP, Wrzaczek M. CRK2 Enhances Salt Tolerance by Regulating Callose Deposition in Connection with PLD α 1. *Plant Physiol*. 2019; 180(4): 2004-2021.
134. Cao L, Wang W, Zhang W, Staiger CJ. Lipid signaling requires ROS production to elicit actin cytoskeleton remodeling during plant innate immunity. *Int J Mol Sci*. 2022; 23(5): 2447.
135. 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(9): 1012-1021.
136. D'Ambrosio JM, Gonorazky G, Sueldo DJ, Moraga J, Di Palma AA, Lamattina L, Collado IG, Laxalt AM. The sesquiterpene botrydial from *Botrytis cinerea* induces phosphatidic acid production in tomato cell suspensions. *Planta*. 2018; 247(4): 1001-1009.
137. Raho N, Ramirez L, Lanteri ML, Gonorazky G, Lamattina L, ten Have A, Laxalt AM. Phosphatidic acid production in chitosan-elicited tomato cells, via both phospholipase D and phospholipase C/diacylglycerol kinase, requires nitric oxide. *J Plant Physiol*. 2011; 168(6): 534-539.
138. 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.
139. Kasparovsky T, Blein JP, Mikes V. Ergosterol elicits oxidative burst in tobacco cells via phospholipase A2 and protein kinase C signal pathway. *Plant Physiol Biochem*. 2004; 42(5): 429-435.
140. Serna-Sanz A, Parniske M, Peck SC. Phosphoproteome analysis of *Lotus japonicus* roots reveals shared and distinct components of symbiosis and defense. *Mol Plant Microbe Interact*. 2011; 24(8): 932-937.

141. Vergnolle C, Vaultier MN, Taconnat ., Renou JP, Kader JC, Zachowski A, Ruelland E. The cold-induced early activation of phospholipase C and D pathways determines the response of two distinct clusters of genes in *Arabidopsis* cell suspensions. *Plant Physiol.* 2005; 139(3): 1217-1233.
142. Genva M, Fougère L, Bahammou D, Mongrand S, Boutté Y, Fouillen L. A global LC-MS²-based methodology to identify and quantify anionic phospholipids in plant samples. *Plant J.* 2023.